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# NEPHRITIS IN ANIMALS.

By Sir JOHN MCFADYEAN.

(*Continued from page* 71.)

IN the previous article on this subject the generally recognised difficulty of making a scientific classification of the different forms of nephritis was mentioned, but any attempt in that direction was temporarily avoided by dealing in the first place with the class in which pyogenic bacteria are demonstrably the cause of the inflammation.

In the other forms of nephritis an etiological basis for classification cannot be employed since there is neither certainty nor general agreement as to their causes. At the present day the classification adopted by human pathologists is based partly on the primary or main site of the lesions, and partly on the course or rate of progress of the disease.

For example, Professor Muir in his text book of Pathology (1925) divides inflammatory conditions of the kidney into the acute, the sub-acute, and the chronic. Under the first of these heads are included acute glomerular nephritis, acute catarrhal nephritis, and acute interstitial nephritis, and under the second sub-acute and chronic glomerular nephritis, or diffuse nephritis, sub-acute catarrhal nephritis, embolic nephritis, and primary renal cirrhosis or fibrosis. The last of these includes primary granular contracted kidney (genuine contracted kidney of German writers), and senile contracted kidney. Renal fibrosis or primary granular contracted kidney is associated with high blood pressure, general arterio-sclerosis, and cardiac hypertrophy, whereas senile contracted kidney, without hypertonic accompaniments, may be regarded as secondary to senile arterial disease. The last two forms of nephritis are further distinguished by certain differences in the character of the alterations in the kidney substance.

On the present occasion the author proposes to deal with the occurrence in animals of cases of nephritis that appear to correspond

with " primary renal cirrhosis or fibrosis " in the above classification, or, to adopt an older term, chronic interstitial nephritis.

## Chronic Interstitial Nephritis.

To provide a basis of comparison, it appears to be necessary to show what are the lesions characteristic of this disease in man.

According to Muir, in *primary granular contracted kidney*, also known as arterio-sclerotic kidney, the granularity of the organ is pretty even in distribution and the colour is usually red. There are important changes in the arterioles, and there may be no evidence of pre-existing proliferic changes in the glomeruli tubules such as occur in the secondary contracted kidney of chronic nephritis. The capsule is distinctly thickened and adherent ; the surface is granular and has a mottled appearance ; the elevated parts are paler, while the depressions have a deep red colour owing to the fact that they correspond with the contracting connective tissue, in which there is considerable vascularity. Sometimes small cysts with colloid or watery contents are present on the surface. The consistency of the kidney is somewhat firm and elastic. On section the cortex is found to be atrophied in varying degree, and the normal markings have disappeared, the parenchyma presenting a confused reddish mottling or stippling. The walls of the arteries are thickened and stand out prominently. This is notably the case in the boundary zone, where the vessels appear to be both increased in size and more numerous, owing to the fact that they are often tortuous and thus appear several times in the section. The medulla also is diminished in thickness, but less proportionately than the cortex, and the pelvic fat is increased in amount to compensate for the general atrophy. The renal arteries and its branches in the hilum are usually the seat of distinct arterio-sclerosis. It is often impossible to show without microscopic examination whether a granular contracted kidney is of the primary or secondary type, and not infrequently the histological changes partake of the characters of both.

Microscopic examination shows in the cortex areas of fibrosis alternating with portions less affected, the former owing to the contraction which occurs correspond to the depressions, and in them the connective tissue is seen to be definitely related to the interlobular arteries. These vessels are tortuous and have thickened walls, which are more or less sclerotic and may show fatty change ; their connective tissue is sometimes hyaline and more distinct arteritis obliterans may be present. All stages of diminution of the lumen down to complete closure of the minute twigs may be seen. The glomeruli are gathered together in the connective tissue around the arteries and are quite irregular in their arrangement. Some of them appear comparatively healthy, while others are sclerosed and changed into small balls of hyaline appearance, enclosed in a thickened and closely applied fibrous capsule. There is no doubt that we have here a picture of a gradual narrowing of the small arterioles and ultimately a cutting off of the blood supply to the glomeruli.

In the connective tissue the tubules are for the most part atrophied and lined with small cubical cells. Patches of lymphocytic infiltration are often present in the fibrous tissue ; on the other hand, the intervening areas, corresponding to the elevations, are composed chiefly of tubules between which there is comparatively little increase of fibrous tissue. The tubules are often dilated, and the epithelium tends to be of the cubical type, but in some of them the epithelial cells are of large size and of distinctly granular appearance, and there may be even small capillary growths in some tubules. These latter changes represent a hypertrophic process of compensatory nature, following

FIG. 1.

Shows development of connective tissue between the tubes in the deeper part of the cortex, and dilatation of the tubes elsewhere.

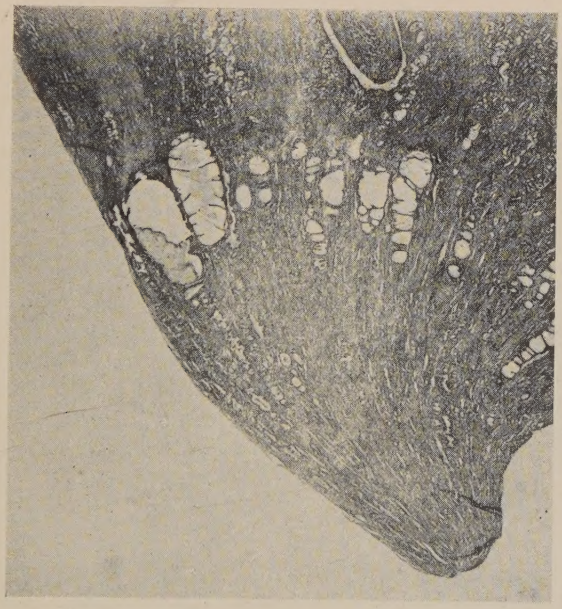

FIG. 2. (CASE I).

Shows medulla and boundary layer. Shows the fibroid condition of these parts and the peculiar cystic dilatation of the tubes.

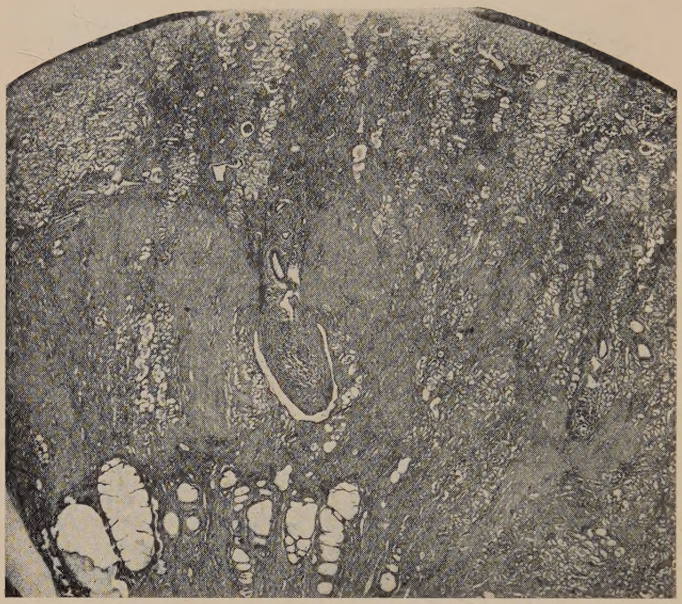

FIG. 3 (CASE I).
Shows alterations in cortex, boundary layer, and medulla.

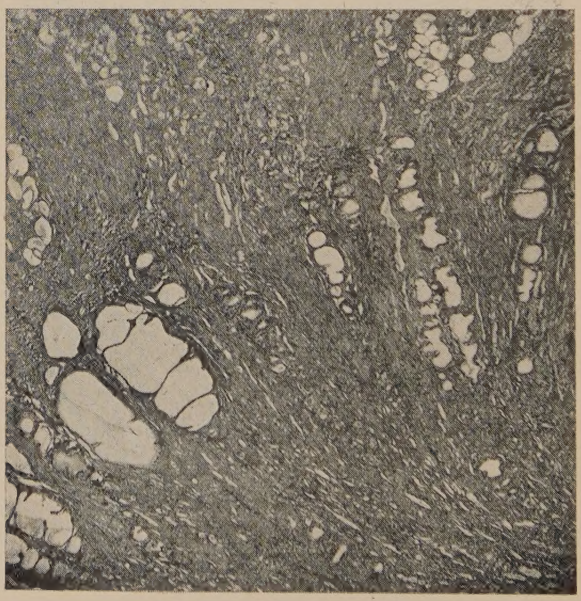

FIG. 4 (CASE I).
Same as Fig. 2.

upon the atrophy of other tubules. There are usually present colloid casts, which are often very abundant in the collecting tubules.

*Senile contracted kidney.* In this form of nephritis the kidneys are diminished in size and may be very small. One is often more shrunken than the other. The capsule is as a rule less thickened and adherent than in the previous type, and when stripped off leaves a surface which is very irregular. There may be depressed areas of a considerable size which have a finely granular or almost smooth surface of a red colour, whilst the elevated portions are paler. Some parts resemble the ordinary granular contracted kidney, but the essential point is that certain portions of the kidney are extremely atrophied, and these are very irregularly distributed. In the atrophied areas there are groups of sclerosed Malpighian bodies, irregularly drawn together along with atrophied tubules, but the overgrowth of the connective tissue is proportionately less than in the forms previously described. Atheroma is commonly present in the large arterial branches, while smaller twigs show much hyaline thickening which leads to diminution of the lumen. There is no doubt that the changes in the kidneys are essentially the result of those in the arteries, and that their irregular distribution is due to the varying degree to which the branches are affected. Much destruction of the kidney substance may occur without distinct symptoms resulting.

In Aschoff's text book of Pathological Anatomy (1928) the account which is given of the macroscopic and microscopic characters of primary granular contracted kidney and senile contracted kidney do not differ in any important respect from those which have just been quoted from Professor Muir, but Aschoff does not regard the latter disease as a form of nephritis. He places it in a group of abnormalities of the kidneys which are determined by an increasing narrowing of the vessels with atrophy as a consequence. With regard to the hypertrophied parts of the tissue in cases of primary contracted kidney, it is said that the cicatricial parts take the form of distinct stripes which can be followed through the whole thickness of the cortex into the medullary substance. The nature of the change in the glomeruli is said to be of importance for diagnosis. Generally these are sharply circumscribed, uniformly hyaline, with occasional remains of nuclei. The capsules are thickened just as in the senile atrophy. The more recent the interstitial changes in the cortex the more abundant is the round-celled infiltration. In older cases the new connective tissue has become sclerotic and encloses the remains of the completely atrophied tubules and the closely compressed hyaline glomeruli.

## Frequency in Animals.

The opinions expressed by veterinary authors concerning the frequency of chronic forms of nephritis in animals vary greatly.

In the text book by Cadiot, Lesbouyries, and Ries (1925) they are said to be relatively common, although it is pretty generally believed that they are rare. They are infrequent in the species which serve for the food of man, and of those in which the individuals are often killed while still young; they are especially frequent in the animals used for work and in the dog and cat. Statistics compiled by Hiyri showed that taking all cases of nephritis the proportion occurring in dogs was 67 per cent.

In their well-known work* Hutrya and Marek distinguish two forms of chronic nephritis, *viz.* (1) a diffuse form, which includes chronic parenchymatous nephritis, chronic mixed nephritis, and diffuse

* Sixth Edition, 1922.

chronic glomerular nephritis ; (2) renal induration or cirrhosis, including the so-called granular and contracted kidney. No opinion is expressed as to the frequency of the diffuse chronic parenchymatous form of the disease, but renal cirrhosis is said to occur very frequently in cattle, dogs, and swine, seldom in horses, and exceptionally in other domesticated animals. The disease is comparatively frequent in old dogs, but has been observed in puppies only a few weeks old. As a rule, this form of nephritis appears to follow an acute diffuse nephritis, especially when the latter has not caused a severe disturbance and has therefore not been diagnosed. The occurrence in animals of a nephritis corresponding with the primary contracted kidney of the human subject is considered unproved, since in the former the association of sclerosis of the renal arterioles has not been observed, although the possible occurrence of such arterial disease in adult dogs cannot be excluded.

Marcone*, after describing in detail a case of nephritis in a horse in which the lesions were of a mixed character and were not in accord with those usually found in so-called cases of chronic interstitial nephritis, goes on to refer to current views regarding the occurrence of chronic nephritis in the domesticated animals. Some authors, he says, affirm that chronic nephritis is a disease often met with in the horse, but this opinion does not appear to be well supported by facts. He quotes statistics compiled by veterinary surgeons belonging to the German Army, which showed that in 1895 only five cases of primary nephritis were observed in the army horses, and in each of these cases the disease proved fatal. Between 1896 and 1900, from two to four cases were observed in each year, or about one case of nephritis in 10,000 patients.

Marcone, as a result of his search of the literature at that date, found some 20 cases recorded under the head of chronic nephritis in horses, and it is obvious that in a large proportion of the cases the naked eye characters of the organs contra-indicated a diagnosis of chronic interstitial nephritis.

In the first place, the present author wishes to state his opinion that cases of chronic interstitial nephritis bearing any resemblance to either primary granular contracted kidney or senile contracted kidney of the human subject are exceedingly rare among the domesticated animals with the exception of the carnivora. He has never encountered such a condition of the kidneys in a horse, and he believes it to be nearly equally rare in the other herbivora and in the pig. This rarity appears to him to be one of the most remarkable facts in comparative pathology.

On the other hand, he is in agreement with the veterinary authors who have recorded the great frequency with which a similar if not identical condition is met with in the dog. The following notes of a few cases, with the help of the accompanying illustrations, may serve to describe the naked-eye and microscopic alterations which are usually present in this form of nephritis.

* Revue Gen. de Med. Vét., Tome 7, 1906, pp. 345-360.

FIG. 5 (CASE I).
A higher magnification of part of Fig. 2.

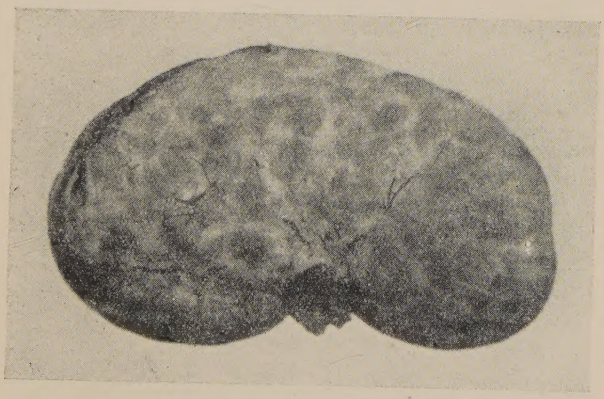

FIG. 6 (Case II).
Kidney. Natural size.

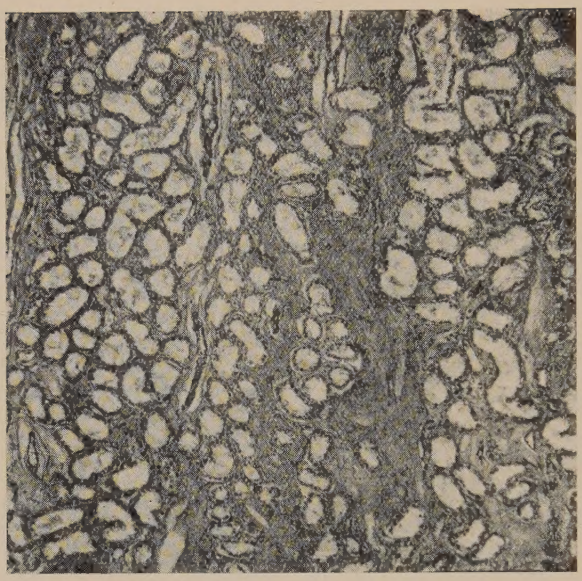

FIG. 7 (CASE II).

Shows increase of connective tissue in a medullary ray and dilatation of the tubes. The section was cut parallel to the normal course of the tubes, and the appearance of cross sections of them suggests that they had become very tortuous.

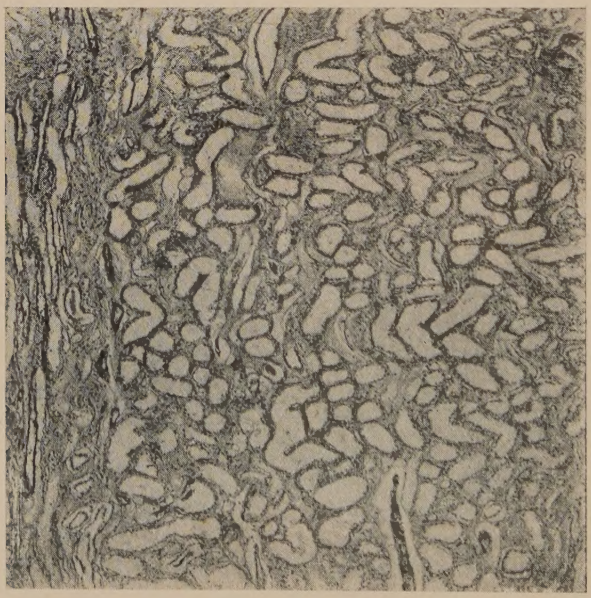

FIG. 8 (CASE II).

Similar to Fig. 7.

*Case I.*

A dog, six years old, breed Dalmatian. Of this animal only the kidneys and a piece of tumour from the animal's prepuce (afterwards proved to be a carcinoma) were forwarded for examination. The sender reported that the animal had been brought to him in order that he might remove the growth, but no operation was performed because the dog was obviously ill, had great thirst, and was passing a large amount of urine. His gait became affected and he developed signs of pneumonia, from which he died.

The kidneys were abnormally small, approximately of the same size, and had a combined weight of 59 grammes. In each case the surface of the organ was not irregular and the capsule was not thickened.

When they were divided mesially the cortex appeared to be reduced in thickness, but its inner limit was not well defined. For the most part it was abnormally pale. The medulla and boundary layer had the usual pale appearance, and it may here be stated that the cysts present in it were not visible, and were not detected until the sections were prepared.

Inspection of the accompanying figures (1, 2, 3, and 4) will serve to give a good idea of the nature of the change in the organ. Dealing in the first place with the cortex, it will be seen in Figs. 1 and 2 that the normal tissue has been in part destroyed and replaced by new connective tissue. Examination of different sections showed that the distribution of this new tissue was very far from regular throughout the cortex. It was most abundant in the deeper part of that layer, and at many places it failed to reach the surface. It stained with only slight intensity with eosin, and was not very richly nucleated. It was in much greater abundance throughout the boundary layer and medulla. (The distribution of this tissue can be followed from its darker shade in the photographs.)

Included in the connective tissue in the cortex there were Malpighian bodies and tubules in various stages of destruction. In the parts not invaded by the new connective tissue the epithelium of the tubes was in a state of fatty degeneration. Slight dilatation of the large collecting tubes was recognisable even at the summit of the renal papilla, but this attained its maximum in the boundary layer, where the distension became very unequal (*see* Figs. 3 and 4). Throughout both boundary layer and medulla many tubes appeared to have been destroyed. Neither in the larger arteries in the boundary layer nor in the cortex was there any evidence of arterio-sclerosis.

The last mentioned fact is an important one, and others are (1) that the fibrosis of the renal tissue affected the boundary layer and medulla to a greater extent than the cortex, and (2) that, although it is impossible to be certain where the fibrosis began, it appeared to be not improbable that it was in the middle part of the kidney substance ; and at any rate, the invasion of the cortex appeared to have extended from below. The cystic dilatation of the tubes is, of course, a striking feature of the changes.

*Case II.*

It is unfortunate that there is no history attaching to this case, except that the kidneys belonged to a Collie dog, and that the animal had a streptococcus abscess in the mesenteric glands. It also suffered from pyorrhœa.

The external appearance of one of the kidneys is shown in Fig. 6. The capsule was irregularly thickened, and the organs were somewhat reduced in volume (Fig. 6 is actual size).

Notwithstanding the irregular surface of the organs and the thickening of the capsule, microscopic examination showed that there was no notable excess of connective tissue in the cortex, but throughout the medulla there

was a distinct increase, associated with compression of the tubules, and at some places irregular cyst formation similar to that shown in Case I. In the boundary layer all the tubes showed moderate dilatation, and were lined by a thin cubical epithelium (*see* Fig. 7). In the cortex the convoluted tubes were all more or less dilated, and lined by an epithelium inclined to columnar in shape. The individual cells were abnormally distinct and the cytoplasm stained to an unusual depth with eosin. There was no evidence of destruction of Malpighian bodies or of the cortical tubules, and for the most part the glomeruli appeared normal and blood corpuscles were recognisable in many of them. In a few, Bowman's capsule was distinctly thickened (*see* Fig. 9).

The branches of the renal artery appeared to be normal.

*Case III.*

The animal in this case was a mongrel dog, rather smaller than a fox-hound. The two kidneys were similar in appearance, and together weighed 1 lb. 3 ozs. Other organs were not sent, and no history was supplied. The surface of each kidney showed shallow pitting. When one of the organs was divided it was found that for the most part the cortex had lost its normal reddish colour, and had a dirty white appearance. A thin stratum under the capsule was nearer the original colour. Some small hæmorrhages were seen in the medulla.

In the cortex in this case there was very little interstitial change, but for the most part the tubes were slightly dilated. At some places there were collections of round mononuclear cells between the tubes, but no actual fibrous tissue.

In the boundary layer this small-celled infiltration was much more extensive, and had involved the compression of some of the tubes and slight dilatation of some of the others (*see* Fig. 13). In the deeper part of the cortex where the small-celled infiltration surrounded Malpighian bodies the latter showed thickening of Bowman's capsule, which appeared to be forming adhesions with the glomerulus (*see* Fig. 14). There was no evidence of arterio-sclerosis in the arteries.

*Case IV.*

The subject in this case was an Airedale dog, which was found dead. No symptoms of serious illness had previously been noted except that there was diarrhœa four days before death, but it lasted only a day. Examination of the urine found in the bladder at post-mortem examination revealed the presence of a considerable quantity of albumen. Both kidneys were notably smaller than normal, but the capsules were not adherent. When a kidney was divided mesially the cortex was seen to be much reduced in thickness (Fig. 20). The normal appearance of its substance was obscured, but in both organs there were whitish areas extending radially, and between these there appeared to be normal, but congested, labyrinth.

Here again the most striking alterations were in the medulla, and they are shown in Figs. 22 and 23. They were of the same nature as those described in connection with the previous case (Case III), but the cystic dilatation of the tubes was more extreme, although the presence of the cysts was not detected until sections were cut.

The cortex also showed alterations similar to those present in Case I. In its deeper part (Fig. 21) it showed, apparently in the position of the medullary rays, collections of moderately dilated tubes filled with homogeneous material, staining pretty deeply with eosin (colloid). Similar material was present in some of the larger cavities in the medulla ; possibly it was present in the majority but had been lost in the process of mounting sections. In the medulla

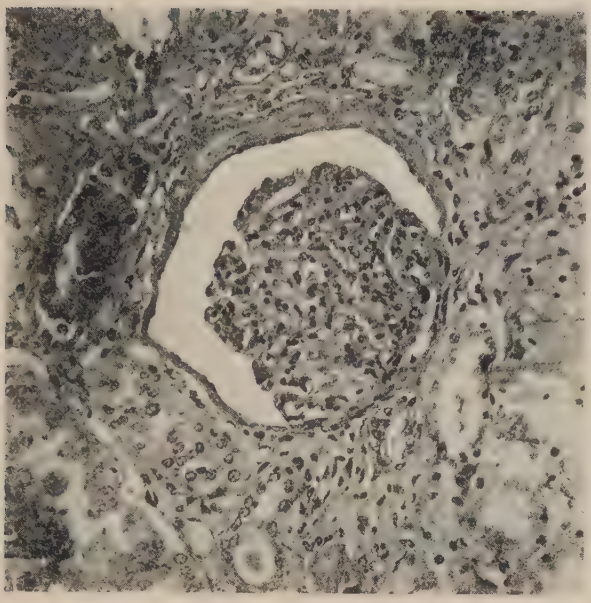

FIG. 9 (CASE II).
Shows thickening of Bowman's capsule and partial collapse of the glomerulus.

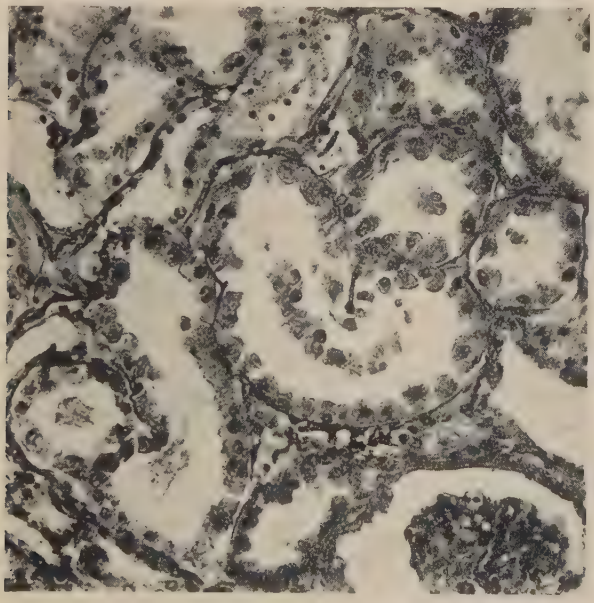

FIG. 10 (CASE II).
Shows dilatation of the convoluted tubes and alteration in the form of the epithelium (*See also* Fig. 27.)

FIG. 11 (CASE III).

Section showing boundary layer, with compression and destruction of tubes by small-celled infiltration.

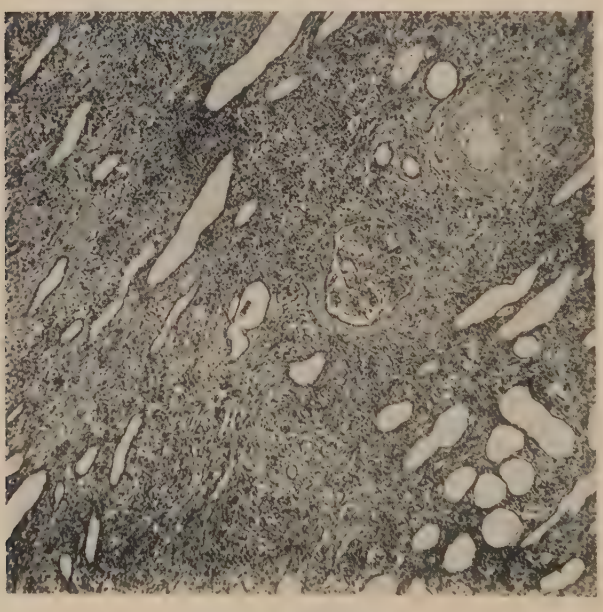

FIG. 12 (CASE III).

Similar to Fig. 11. Note thickening of Bowman's capsule of the Malpighian body.

the gross dilatation of the tubules began rather abruptly in the boundary layer, and became less in passing deeper, but there was moderate dilatation even in the renal papilla.

The renal arteries appeared normal.

*Case V.*

This was a mongrel dog, about the size of an Airedale. The kidneys were of approximately the same size and both were distinctly smaller than normal. The capsule was not distinctly thickened, and it could be stripped off without lacerating the surface of the cortex. The apppearance of the stripped cortex is shown in Fig. 24, which is natural size. The surface was slightly uneven and of a greyish red colour, but dotted with lighter tubercle-like points.

The cortex on microscopic examination showed changes similar to those described in Case I. The greater part of the medulla showed the appearance presented in Fig. 25. At some places the amount of fibrous tissue between the dilated tubes was much more extensive than it is in Fig. 25, but it gave little evidence of active growth, being sparsely nucleated and almost homogeneous. A few tubes contained colloid material. Arteries normal.

The outstanding microscopic change in the kidneys in the present case was the very remarkable degeneration and detachment of the lining epithelium in the dilated tubes of the medulla (Fig. 25). The appearance is likely to suggest post-mortem disintegration of the epithelium, but that can be dismissed as the dog had been killed and the kidneys were secured while still fresh. It ought to be added that an identical appearance has been found in other cases of nephritis in dogs which it is not necessary to describe here.

The writer is of opinion that the alterations which are described and illustrated in the preceding pages are found in practically all cases of indurated or contracted kidney in the dog. The process is obviously a chronic one, and leads in the cortex to destructive changes not obviously different from those found in the so-called primary granular or contracted kidney in man, but as a rule without the arterial changes which are generally believed to be the main causal factor in the human subject. Taking the whole kidney substance into consideration, what dominates the microscopic picture is the changes in the medulla, *viz.*, the increase in the connective tissue of that part, the partial destruction of the tubes, and the remarkable dilatation of those that remain. So far as one can judge from the text books, changes similar in nature and extent are not usually present in either of the forms of contracted kidney in man.

Basset and Chaussé* appear to have been the first to call attention to the frequent occurrence of these medullary cysts in cases of chronic nephritis in the dog. They placed them in a separate category from the larger cysts that are not rarely found in the cortex in otherwise apparently normal kidneys, and also from the numerous smaller cysts that are sometimes present in the cortex in chronic nephritis. They ascribed the formation of the cysts to a neoplastic growth of the epithelium, involving an actual multiplication of the cysts—a process of "neoformation conjunctivo-epitheliale."

* Rev. Gen de Med. Vét., 1906, p. 465.

They called attention to the short, spur-like, inward projections of the wall of some of the cysts (*see* Fig. 23) and described these as "veritables papilles," the formation of which they ascribed to inward growth of buds of connective tissue.

The adenomatous nature of the alterations in the medulla was also accepted by Ball and Roquet,* who cited the new growth as another example "showing once more that the trench which science and tradition has dug between tumours and inflammatory lesions is not so deep as has generally been supposed."

With that view of the origin and nature of the cysts the present author finds himself unable to agree. A glance at the figures in this article will show that the formation of the cysts can be explained by the simpler supposition that they result from compression or obstruction of the collecting tubules, with consequent dilatation from the increased tension of their accumulating contents.

The dilatation is very unequal in different tubules and even at different points in the same tubule, which is no doubt due to the varying resistance to distension offered by the walls of the tubules or the interstitial tissue. Sometimes, especially in the medullary rays and the boundary layer, the normally straight tubules become tortuous before they are much dilated. The stretching of the cyst walls under the increased pressure of their contents generally causes flattening of the epithelial lining, but sometimes the cells show little deformity, and in the larger cysts there may be two or three layers of cells.

The clue to the nature of the alteration may be lost where the process is most advanced, but in the early stage (as in Fig. 18) the production of the cysts by dilatation of the pre-existing normal tubules is manifest.

Lastly, it may be observed that the apparent formation of in-growths or solid buds, with central connective tissue, from the wall of some of the cysts, is not acceptable as evidence that one is dealing with an adenoma or that cysts are multiplied by the formation of such papillæ.

But what appears to be even a more serious objection to the adenomatous theory of the origin of these medullary cysts is the fact that they are found in both kidneys and to approximately the same extent, and that the entire medulla is involved in the process of cyst-formation. Nothing comparable to that has ever been observed in connection with the growth of a genuine neoplasm either in the kidney or in any other organ.

(*To be continued.*)

* Jl. de Med. Vét., 1912, p. 334.

FIG. 13 (CASE III).
The central part of the Figure shows dilatation of the tubes in a medullary ray. On either side destructive changes are seen in the labyrinth.

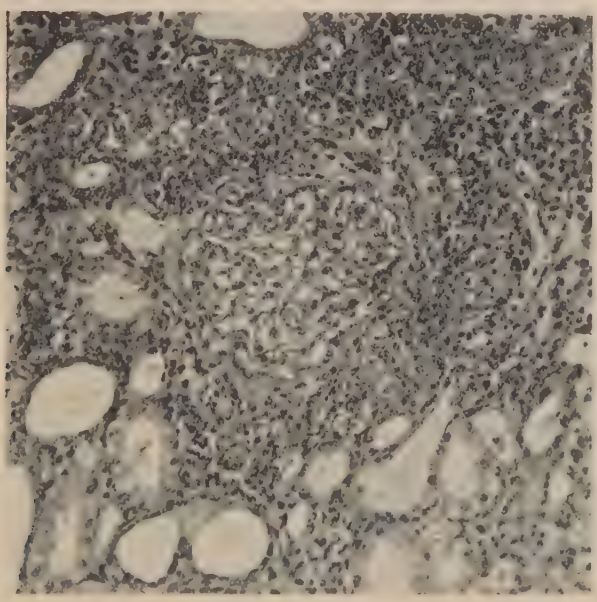

FIG. 14 (CASE III).
Shows infiltration with small uninuclear cells around a Malpighian body. Bowman's capsule is thickened and adherent to the glomerulus.

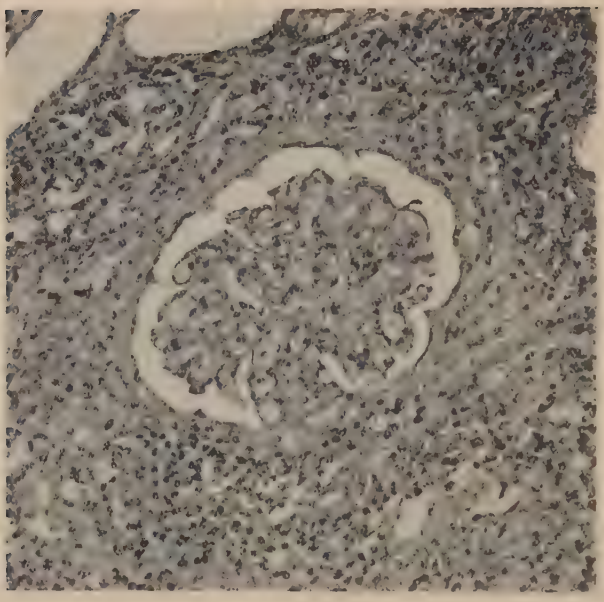

FIG. 15 (CASE III).
Similar to Fig. 14. Shows torn adhesions between the glomerulus and the capsule.

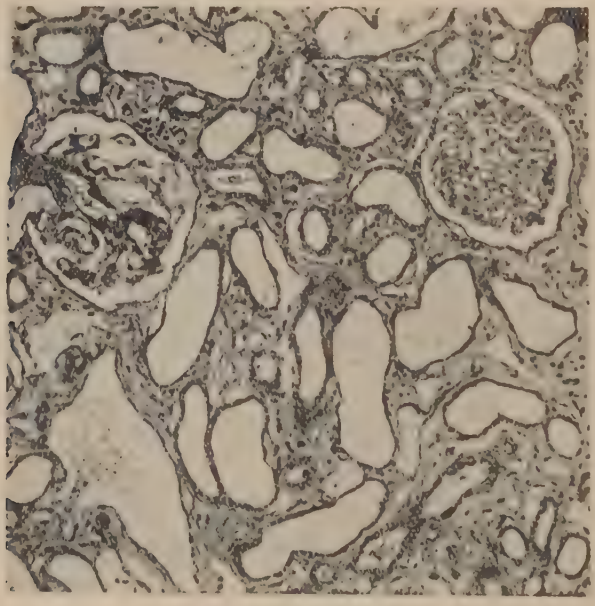

FIG. 16 (CASE III).
Section near the junction of the medulla and boundary layer. Shows the dilatation of the tubes and the compression of the epithelium.

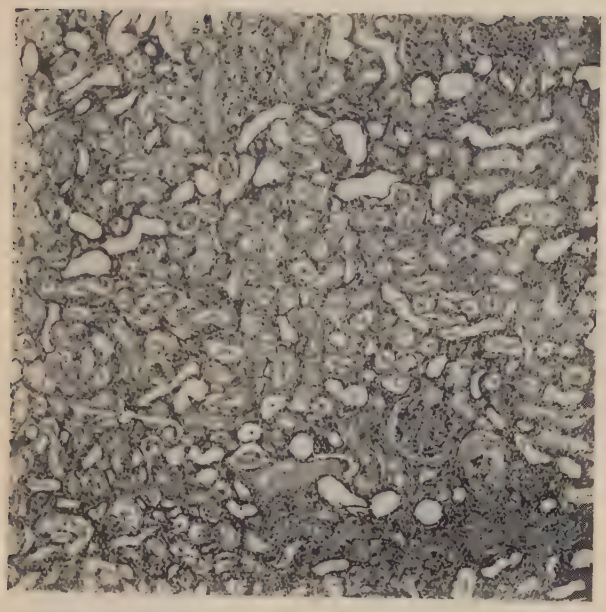

FIG. 17 (CASE III).
From the most superficial part of the cortex.

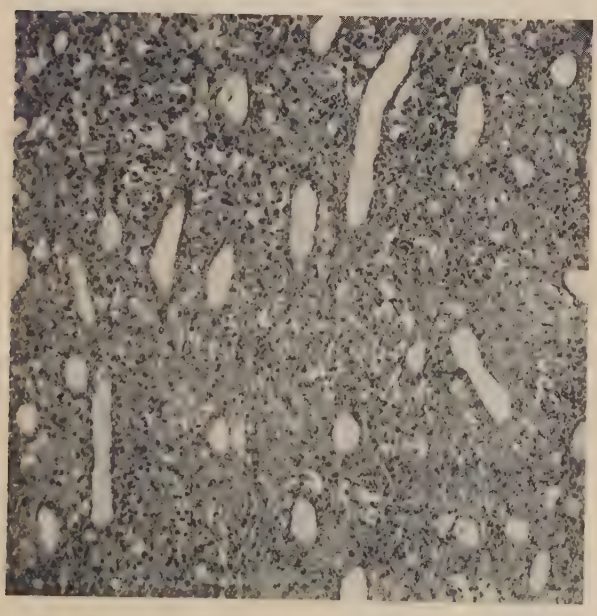

FIG. 18 (CASE III).
From the outer part of the boundary layer, showing small-celled infiltration, with destruction of some tubes and dilatation of others.

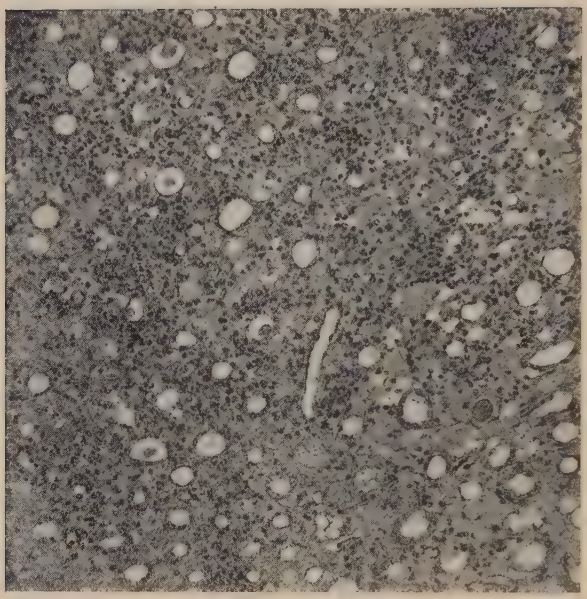

FIG. 19 (CASE III).

From the deeper part of the medulla. Cut nearly at right angles to the course of the tubes. Shows small-celled infiltration. Some of the tubes are partially occupied by colloid material and are dilated.

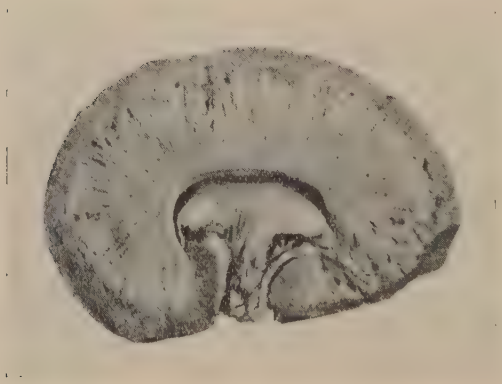

FIG. 20 (CASE IV).

Divided Kidney. Photo taken after fixation in 5 per cent. formalin. Shows great reduction in thickness of the cortex. Cysts are visible in the medulla.

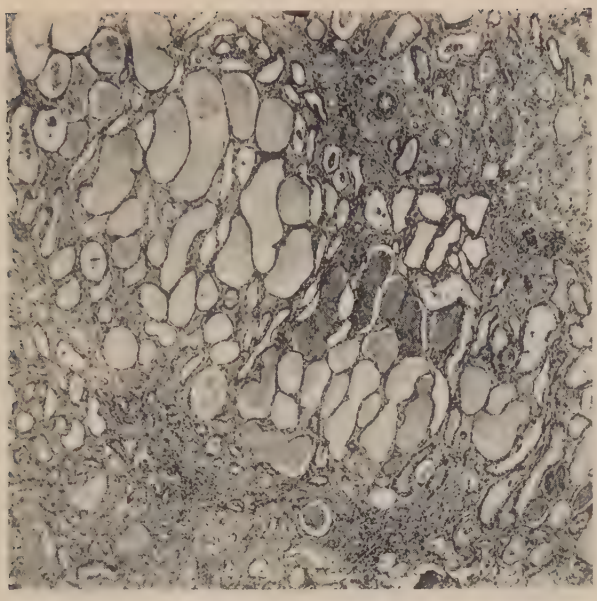

FIG. 21 (CASE IV).
The centre shows an oblique section through a medullary ray, with dilated tubes filled with colloid. The cortex above and below shows cirrhotic changes.

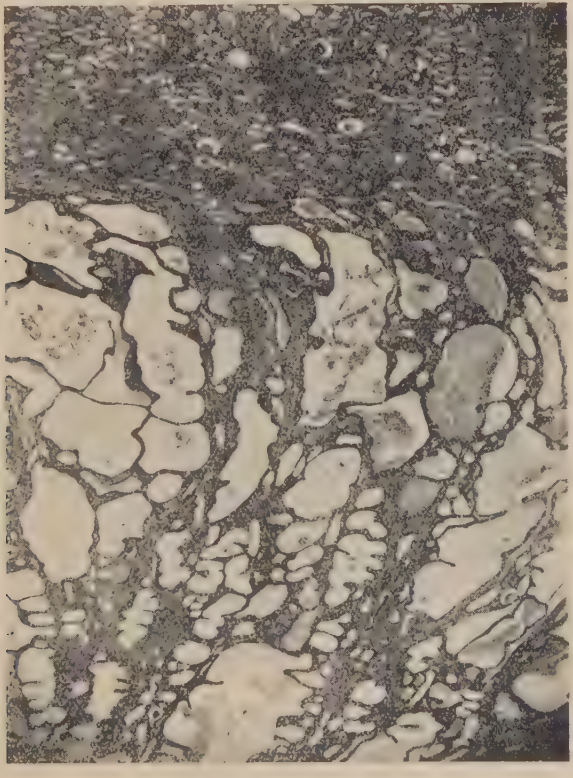

FIG. 22 (CASE IV).
Shows extensive cystic dilatation of collecting tubes in medulla next the cortex.

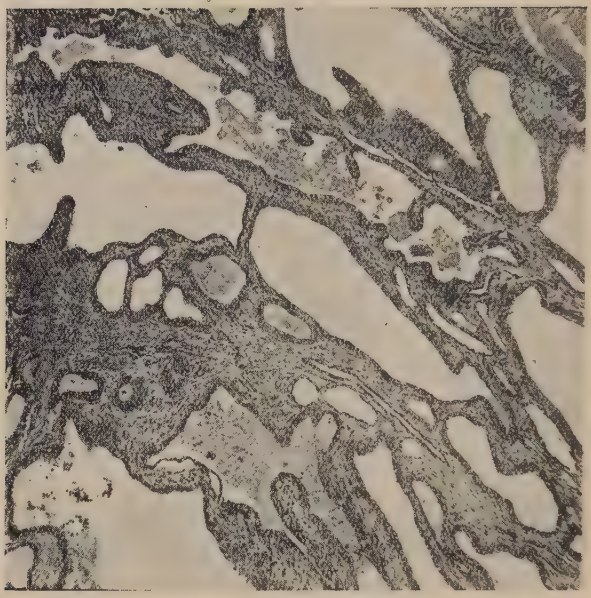

FIG 23 (CASE IV).
Part of Fig. 22 more highly magnified.

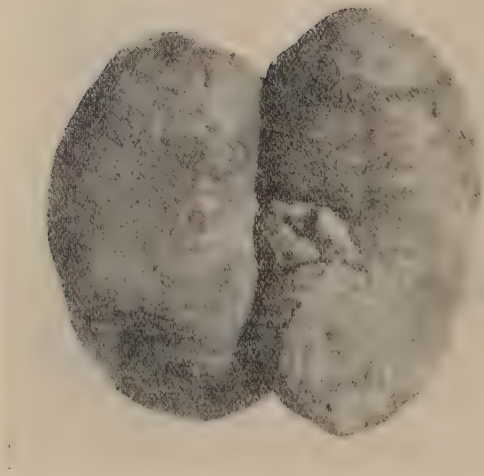

FIG. 24 (CASE V).
Kidney. Actual size after fixation.

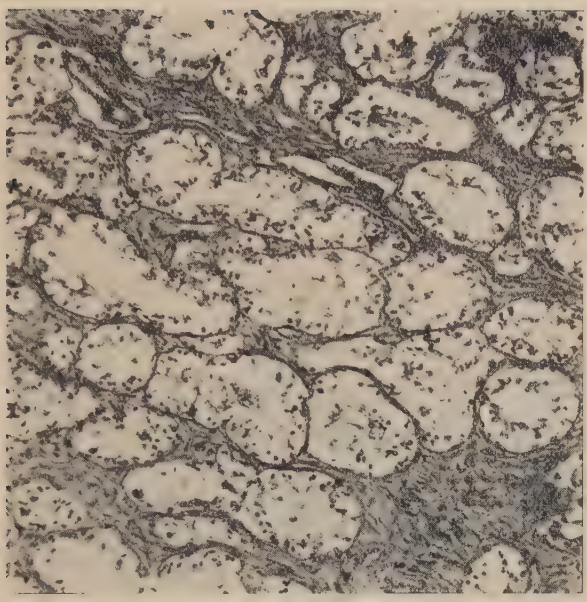

FIG. 25 (CASE V).
Shows peculiar degeneration and detachment of the epithelial lining of the dilated tubes in the medulla.

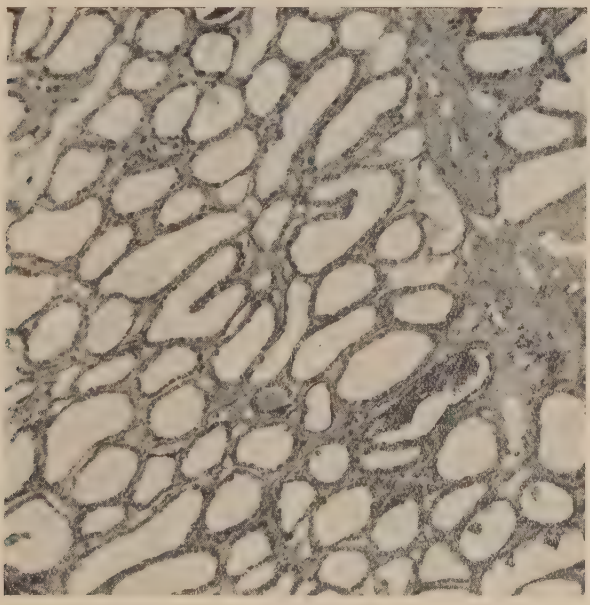

FIG. 26.
From a case not described in the text. Shows early dilatation of the collecting tubes and flattening of their epithelium.

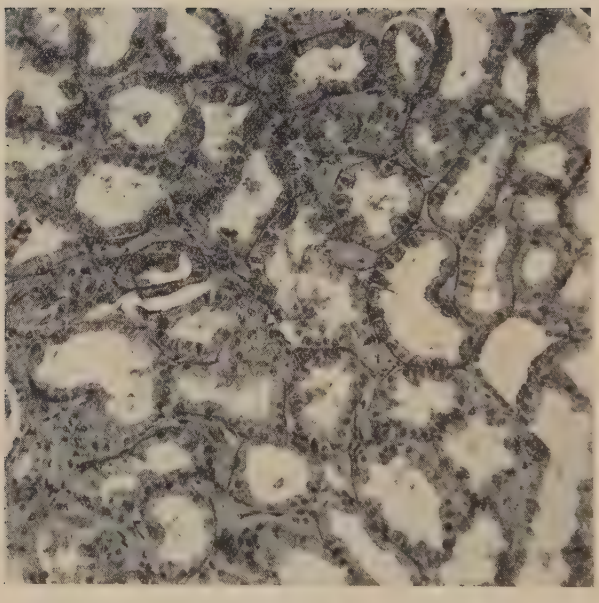

FIG. 27.

From the same Kidney as Fig. 26. A collection of dilated convoluted tubes in a cortex that showed fibrotic interstitial changes. Suggestive of regeneration or repair.

# A BACTERIOLOGICAL INVESTIGATION OF CASES OF TUBERCULOSIS IN FIVE CATS, SIXTEEN DOGS, A PARROT, AND A WALLABY.

By A. W. Stableforth, B.Sc., M.R.C.V.S., D.V.S.M.

(*Continued from page* 108.)

The methods employed in dealing with these cases were similar to those used in the examination of equine material. (*See* p. 92.)

It was suspected as long ago as 1839 (Malin) that dogs could become affected from contact with cases of human tuberculosis and many single instances of such believed infection were reported (Nocard, Bourgougnon, Brusasco, Johne, and Cadiot), but not until well into the present century was definite bacteriological evidence obtained. Investigations of cases in this country have been very few. Two cases in dogs, from which human bacilli were isolated, were reported by the Royal Commission on Tuberculosis (A. S. Griffith and F. Griffith) and one case of infection with human bacilli by A. S. Griffith (1928), who also notes that G. S. Wilson had isolated bovine bacilli from the mesenteric gland of a five months old puppy. A. S. Griffith (1928) also summarises the investigations carried out in other countries.

The case of tuberculosis in the parrot is believed to be the first example of natural infection in which definite evidence of the human type of the bacillus has been obtained.

It is further established that tuberculosis in cats is usually of bovine origin.

The wallaby must be added to the list of animals which can become infected with bacilli of avian origin.

## Cat 1.

This animal showed at post-mortem a large lesion on the posterior surface of the left lobe of the liver, 5 by 4 by 3 cm., yellow and firm. One omental gland was much enlarged and caseated. The mesenteric, bronchial, and mediastinal glands were enlarged only.

*Bacteriological Examination.*

Acid-fast bacilli were found to be very numerous in the liver lesion and the omental gland.

A culture was obtained direct and proved to be of definitely dysgonic type. Growth was poor on glycerine media at first, but in later generations appeared about equal to that on plain media. Glycerine agar never showed more than a scanty growth ; no growth was obtained on glycerine potato, but on plain potato a few tiny pearly colonies were produced. Growth on glycerine broth never extended far and was of a very thin filmy type, showing no thickening.

The virulence of this strain was slightly lower than that of most bovine strains for guinea-pigs, and distinctly lower for rabbits. However, all except one animal died eventually and all showed tuberculous lesions typical of a bovine strain, though more massive in character than usually follows the

inoculation of a strain of high virulence. It is particularly striking that animals receiving the intravenous doses survived so long.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit ... | 1900 | 1550 | 10·0 mg. subcut. | Died 118 | A.T. |
| | 2150 | 2600 | 0·1 | Killed 186 | A.T. |
| | 3000 | 1700 | 1·0 intrav. | Died 31 | A.T. |
| | 2300 | 1900 | 0·01 | 91 | A.T. |
| Guinea-pig. | 745 | 580 | 10·0 subcut. | 86 | A.T. |
| | 620 | 540 | 10·0 | 90 | A.T. (*a*) |
| | 740 | 595 | 0·1 | 167 | A.T. (*c*) |
| | 485 | 500 | 0·1 | 119 | A.T. (*b*) |

(*a*) Liver and spleen enormous, showing very large caseated areas.
(*b*) All glands greatly enlarged, spleen and liver very large and caseated.
(*c*) All glands, spleen, and liver enormous and caseated.

## CAT 2.

The animal showed advanced generalised infection; the lungs were riddled with small yellow nodules, 2-3 mm. in diameter each, with a tiny caseous centre. The bronchial glands formed a mass about 1·5 cm. in diameter. The liver showed about 30 lesions up to 1 cm., firm and yellowish. The mesenteric glands were enlarged to form an irregular white mass about 4 cm. in diameter, soft in consistence, and appearing to be the seat of primary infection.

*Bacteriological Examination.*

Bacilli were rare and a culture was obtained through a guinea-pig which died at 39 days. Growth was very dysgonic and always very thin, often even after prolonged incubation, so thin that a growth could easily be overlooked; isolated colonies were particularly small and flat.

It was of bovine virulence; one rabbit, however, which received 0·01 mg. intravenously survived 73 days before dying with typical lesions.

ANIMAL INOCULATIONS.

| *Animal.* | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|
| Guinea-pig. | Fresh mesenteric gland. | Died 39 | A.T. |
| *Quantitative inoculations* with Besredka culture 378 days later. | | | |
| Rabbit ... | 10·0 mg. subcut. | Died 64 | A.T. |
| | 1·0 | 83 | A.T. |
| | 0·01 intrav. | 21 | A.T. |
| | 0·01 | 73 | A.T. |
| Guinea-pig. | 10·0 subcut. | 34 | A.T. |
| | 1·0 | 36 | A.T. |
| | 0·1 | 51 | A.T. |

### Cat 3.

The only lesions in this animal were enlarged mesenteric glands, caseated throughout.

*Bacteriological Examination.*

Acid-fast bacilli were rare, rather long and beaded.

A culture, obtained through a guinea-pig, proved to be of moderately dysgonic type. Initial cultures were obtained both on glycerine egg and on plain egg and subsequent generations did not appear to be affected by glycerine. On glycerine agar a thin grey layer was produced, a little thickened at places. Potato was not used.

It was of average bovine virulence.

Animal Inoculations.

| *Animal.* | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|
| *Original inoculations.* | | | |
| Guinea-pig. | Fresh gland. | Killed 21 | E.T. |
| | Antiformined gland. | Died 76 | A.T. |
| Rabbit ... | — | | A.T. |
| | | 112 | A.T. |
| *Quantitative inoculations* with Besredka culture 233 days later. | | | |
| Rabbit ... | 10·0 mg. subcut. | Died 62 | A.T. |
| | 1·0 | 62 | A.T. |
| | 0·1 | 102 | A.T. |
| | 0·01 intrav. | 20 | M.T. (*a*) |
| Guinea-pig. | 10·0 subcut. | 53 | A.T. |
| | 1·0 | 36 | A.T. |
| | 0·1 | 46 | A.T. |

(*a*) Miliary lesions in lungs, liver, and kidneys, those in the lungs being up to 1 mm. with a deeply congested margin and caseated central point. The animal was severely infected with coccidia.

### Cat 4.

This case was peculiar in that macroscopic lesions were present in the pancreas only, that organ being 23 cm. in length when spread out and from 1-3 cm. in diameter. It was attached to the duodenum for the first 8 cm., and its free end then appeared to be reflected towards the spine. It was riddled throughout with rounded greyish-white or greyish-yellow nodules, $\frac{1}{2}$ to 4 mm., which were generally spherical but some of the larger lesions were disc-like. On section the smaller nodules appeared grey and homogeneous, and the larger ones had a tiny caseous centre.

*Bacteriological Examination.*

Acid-fast bacilli were not rare and were slender and moderately long. A culture was obtained from the pancreas, and also one from a guinea-pig, which had been inoculated with material from the mesenteric glands 64 days previously, and which when killed showed progressive lesions. The two

strains were similarly dysgonic, and grew about equally well on plain and glycerine media.

This virus (pancreatic strain) was highly virulent for both rabbits and guinea-pigs.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Fresh pancreas. | Died 14 | E.T. |
| Rabbit ... | | | | 190 | A.T. |
| Guinea-pig. | | | Antiformined pancreas | 20 | Neg. |
| | | | | 30 | Neg. |
| Guinea-pig. | | | Fresh mesenteric gland | 12 | Neg. |
| Guinea-pig. | | | | Killed 64 | E.T. |
| Rabbit ... | | | Antiformined pancreas | Killed 190 | Neg. |
| *Quantitative inoculations* with B esredka culture 437 days later. | | | | | |
| Rabbit ... | 1900 | 1400 | 10·0 mgm. subcut. | Died 37 | A.T. |
| | 1900 | 1100 | 0·1 | 70 | A.T. |
| | 1900 | 1750 | 1·0 intrav. | 19 | A.T. |
| | 2200 | 1600 | 0·01 | 36 | A.T. |
| Guinea-pig. | 545 | 500 | 10·0 subcut. | 14 | E.T. (*a*) |
| | 700 | 650 | 10·0 | 14 | E.T. (*b*) |
| | 625 | 390 | 0·1 | 33 | M.T. (*c*) |
| | 675 | 455 | 0·1 | 49 | A.T. (*d*) |

(*a*) and (*b*) Lesions markedly progressed for short intervals after inoculation.
(*c*) Spleen 1/300th body weight. Liver 1/25th.
(*d*) Spleen 1/200th body weight. Liver 1/10th.

## CAT 5.

The animal was a young male tortoiseshell.

*Post-mortem Examination.*

Very numerous rounded, greyish lesions were present in both lungs and varied from a pin-head to 4-5 mm., the larger ones having a small central caseated point. Section showed that in many places the lung was practically solid, the tissue still crepitant, deeply congested. Pleurisy was present, and particularly on the left posterior lobe. The left auricle of the heart showed five greyish tubercles. There were no lesions in the bronchial, mediastinal, or tracheal glands, and no abdominal lesions.

*Bacteriological Examination.*

Acid-fast bacilli were fairly numerous and distinctly long.

A strain obtained through a guinea-pig proved to be dysgonic and slightly inhibited by glycerine, except on glycerine egg, on which it grew slightly better than on plain egg. Small growth was obtained on plain potato but none on glycerine potato ; a very scanty growth was once obtained on glycerine agar. Isolated colonies were particularly flat and often stellate.

It was highly virulent for both rabbits and guinea-pigs.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| Guinea-pig. | | | Fresh lung. | Killed 25 | E.T. |
| Rabbit .... | | | Antiformined lung. | Died 64 | A.T. |
| *Quantitative inoculations* with Besredka culture 437 days later. | | | | | |
| Rabbit ... | 2000 | 1500 | 10·0 mg. subcut. | Died 32 | A.T. |
| | 2500 | 2000 | 0·1 | 90 | A.T. |
| | 1800 | 1500 | 1·0 intrav. | 18 | A.T. |
| | 2250 | 1750 | 0·01 | 24 | A.T. |
| Guinea-pig. | 610 | 620 | 10·0 subcut. | 13 | E.T. (*a*) |
| | 535 | 465 | 10·0 | 36 | A.T. (*b*) |
| | 510 | 315 | 0·1 intrap. | 18 | A.T. |
| | 560 | 480 | 0·1 | 13 | E.T. |

(*a*) Markedly advanced for time.
(*b*) Spleen 1/400th of body weight, liver one sixteenth.

DOG 1.

A much enlarged mesenteric gland, from an Irish Wolfhound, nine months old, was the only lesion received. It was yellowish in colour, and when cut contained very soft caseous material, which was teeming with acid-fast bacilli. As far as could be ascertained this was the only lesion present.

*Bacteriological Examination.*

No direct strain was obtained as decomposition was advanced, but a strain was isolated from each of two guinea-pigs which had been inoculated with antiformined material.

Growth was always dryish and very thin, but was about equal on plain and glycerine media; no growth was obtained on glycerine agar, and that on glycerine potato was scanty.

The virulence for rabbits was distinctly higher than that of most other viruses examined, and in this connection it is interesting to recall the fact that 625 days had elapsed since the original material was obtained.

ANIMAL INOCULATIONS.

| *Animal.* | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|
| *Original inoculations.* | | | |
| Rabbit ... | Antiformined material. | Died 120 | A.T. |
| | | 154 | A.T. |
| Guinea-pig. | | 81 | A.T. |
| | | 87 | A.T. |
| *Quantitative inoculations* 625 days later with Besredka culture. | | | |
| Rabbit ... | 10·0 mg. subcutaneously. | Died 43 | A.T. |
| | 1·0 | 55 | A.T. |
| | 0·1 | 57 | A.T. |
| | 0·01 intravenously. | 18 | A.T. |
| | 0·01 | 22 | A.T. |
| Guinea-pig. | 10·0 subcutaneously. | 22 | A.T. |
| | 1·0 | 32 | A.T. |
| | 0·1 | 37 | A.T. |
| | 0·1 | 43 | A.T. |
| | 10.0 intraperitoneal. | 11 | M.T. |
| | 0·1 | 13 | M.T. |

## Dog 2.

The animal was a large male mongrel, which was reported to have been showing respiratory distress for a few days only ; no other history was available.

*Post-mortem Examination.*

The lungs contained fairly numerous firm nodules up to ·5 cm. in diameter ; pleural lesions, either in the form of small greyish-white patches or actual grapes, were widespread on the diaphragm, ribs, sternum, and pericardium. The mediastinal tissue showed numerous whitish or pale-yellow lesions up to 3 mm. diameter and of firm character ; in some places these had coalesced to form larger irregular lesions. A few whitish nodules, hard and difficult to crush, were present in the liver. Each kidney showed three or four whitish projecting nodules in the cortex and one or two wedge-shaped lesions extending to the medulla. The abdominal glands appeared normal.

*Bacteriological Examination.*

Acid-fast bacilli were rare in all lesions, but a few were found in a liver lesion.

Direct cultures were obtained, and grew profusely on all differential media and particularly on those containing glycerine ; the latter fact was well brought out in serial cultures. They were, however, distinctly slower than most eugonic strains.

From the table it will be seen that the culture, whilst virulent for the guinea-pig, was practically avirulent for the rabbit subcutaneously and intravenously. The lesions in the animal which had received 0·01gm. intravenously were of the shot-like type, showing no caseation nor any reaction in the surrounding tissues.

Animal Inoculations.

| *Animal.* | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|
| *Original inoculations.* | | | |
| Rabbit ... | Fresh material. | Killed 204 | Local. |
| Guinea-pig. | | Died other disease. | |
| do. | do. | do. | |
| *Quantitative inoculations* with Besredka culture 477 days later. | | | |
| Rabbit ... | 10·0 mg. subcutaneously. | Killed 202 | Neg. |
| | 1·0 | 182 | |
| | 0·1 | 211 | Local. |
| | 0·01 intravenously. | 89 | S.T. (*a*) |
| Guinea-pig. | 10·0 subcutaneously. | Died 51 | A.T. |
| | 1·0 | 64 | A.T. |
| | 0·1 | 84 | A.T. |

(*a*) Tiny shot-like lesions in lungs only.

## Dog 3.

The animal showed advanced lesions, which were confined to the thorax. Both lungs were riddled with lesions of the usual type, from 2 mm. up to 1 cm.; some of the larger ones were softened and on section showed cavities. The thoracic glands were also affected.

*Bacteriological Examination.*

The strain was obtained after passage through a guinea-pig, and after a few generations on artificial media was recognised to be of typically eugonic type.

Virulence for rabbits was very low and guinea-pigs inoculated with it survived an unusually long time and at death showed very large lesions but not of caseo-necrotic type.

It thus appeared to be of human type.

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Antiformined lung. | Died 44 | A.T. |
| Rabbit ... | | | | Killed 180 | Neg. |
| *Quantitative inoculations* with B esredka cultures 598 days later. | | | | | |
| Rabbit ... | 2100 | 2000 | 10·0 mg. subcut. | Killed 220 | M.T. (*a*) |
| | 2360 | 1300 | 0·1 " | 209 | S.T. (*b*) |
| | 2200 | 2800 | 1·0 intrav. | 220 | S.T. (*c*) |
| | 1600 | 1300 | 0·01 " | 209 | Neg. |
| Guinea-pig. | 605 | 500 | 10·0 subcut. | Died 97 | A.T. (*d*) |
| | 560 | 580 | 0·1 " | 125 | A.T. (*e*) |
| | 665 | 760 | 0·1 " | 194 | A.T. (*f*) |

(*a*) Moderate number of small lesions of inactive type in the lungs.
(*b*) Local and few small subpleural lung lesions, edges solid and in some cases caseous.
(*c*) Few chronic lesions in lungs.
(*d*) Spleen 1/20th of body weight. Liver 1/9th.
(*e*) Spleen and liver very large, caseation not marked in organs or glands.
(*f*) Spleen 1/130th of body weight. Liver 1/10th.

Dog 4.

The animal was a crossbred terrier, aged four years, which had been losing condition for some time and had developed anorexia and a paroxysmal cough.

Tuberculin had been applied subcutaneously, intradermically, and endericmally, and repeated at forty-eight hours, with negative results. During the next two months respiratory distress increased and the cough became more pronounced. At the end of that period the tuberculin test was again used in a similar manner with negative results, and the animal was destroyed.

*Post-mortem Examination.*

Numerous tuberculous areas were present throughout both lungs, many being quite small and of firm character, but in the right lung were five cavities, the largest of which was about 4 cms. in diameter; they were ragged and irregular in character and filled with a frothy blood-stained fluid. The bronchial and mediastinal glands were enlarged.

Pericarditis was advanced, the pericardium being adherent in its upper third whilst fluid of a sero-fibrinous character distended the lower two-thirds; the inner surface of the sac was coated with a brownish-green fibrinous deposit.

The liver and kidneys were extensively affected.

*Bacteriological Examination.*

Acid-fast bacilli were found by microscopic examination in the pericardium, lung, and kidney.

Rabbits and guinea-pigs were inoculated and a strain was obtained from one of the latter. It was of dysgonic type, producing a thin layer of growth or of small colonies, and developing about equally well on plain and glycerine media.

Growth on glycerine broth was better than that of most bovine strains. The first inoculum, from broth placed at the bottom of a serum tube, slowly extended and at the end of two months and half covered the flask with a thin greyish, nearly smooth layer, the original inoculum having by that time distinctly thickened and sunk. Material transferred to a second flask grew well, producing quickly a thin greyish-white layer with a few white lines and excrescences.

When tested on rabbits this strain was found to have the high virulence of a bovine strain.

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | | *Result.* |
|---|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | | |
| *Original inoculations.* | | | | | | |
| Guinea-pig. | | | Fresh. | Died | 31 | A.T. |
| | | | | | 45 | A.T. |
| Rabbit ... | | | | | 76 | A.T. |
| *Quantitative inoculations with Besredka culture 437 days later.* | | | | | | |
| Rabbit ... | 1900 | 1000 | 10·0 mg. subcut. | Died | 56 | A.T. |
| | 2360 | 2000 | 0·1 | | 96 | A.T. (*a*) |
| | 1700 | 1100 | 1·0 intrav. | | 17 | A.T. |
| | 1900 | 1500 | 1·0 | | 22 | A.T. |
| | 2000 | 1500 | 0·01 | | 47 | A.T. |
| Guinea-pig. | 750 | 455 | 10·0 subcut. | | 32 | A.T. (*b*) |
| | 460 | 360 | 10·0 | | 32 | M.T. (*c*) |
| | 678 | 600 | 0·1 | | 74 | A.T. (*d*) |
| | 395 | 370 | 0·1 | | 81 | A.T. (*e*) |

(*a*) Spleen greatly enlarged and showed firm tumour-like lesions up to 2 cm. in diameter.
(*b*) Spleen 1/150th of body weight. Liver 1/30th. Both caseo-necrotic.
(*c*) Spleen 1/300th of body weight and showing grey firm lesions about 2 mm. diameter. Liver 1/40th.
(*d*) Spleen 1/100th body weight. Liver 1/12th. Both caseo-necrotic.
(*e*) Spleen 1/130th body weight. Liver 1/7th. Both caseo-necrotic.

## Dog 5.

The animal was a male bulldog, about three or four years old, belonging to a private family with no history of tuberculosis. It had been ill for some weeks ; anorexia was complete, emaciation advanced, temperature 99°-99·6° F.

Tests with 2 mm. tuberculin opthalmically and 1 cc. subcutaneously had been negative. Shortly after the latter test the animal collapsed.

*Post-mortem Examination.*

Each lung presented numerous consolidated areas, not softened ; the bronchial glands were enlarged and contained tubercles. Pericarditis was the

most pronounced lesion, the inner surface of the pericardium being firmly adherent to the epicardium in the upper third, whilst a small amount of fluid was present in the lower part. The walls were covered with a greyish membrane showing small whitish projections easily scraped off.

The liver was markedly enlarged, with numerous yellowish-white lesions varying in size from 1 mm. to 2 cm., many of the larger areas showing a depressed centre.

Both kidneys showed a number of greyish-white slightly raised cortical lesions up to 1 cm. in diameter.

*Bacteriological Examination.*

Acid-fast bacilli were fairly numerous in the pericardium and kidneys. A culture, obtained through a guinea-pig inoculated with pericardial material, presented all the characters of a definitely eugonic strain and was of low virulence for rabbits.

The virus thus appeared to be of human type.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit .. | | | Fresh. | Killed 186 | Neg. |
| Guinea-p . | | | | Died (epizootic) | E.T. |
| | | | | | E.T. |
| *Quantitative inoculations with Besredka culture 372 days later.* | | | | | |
| Rabbit ... | 1700 | 1600 | 10·0 mgm. subcut. | Died 26 | Local. |
| | 2400 | 2000 | 0·1 | Killed 286 | S.T. (*a*) |
| | 2000 | 1800 | 1·0 intrav. | Died 107 | A.T. (*b*) |
| | 2400 | 1500 | 1·0 | 27 | S.T. (*c*) |
| | 1700 | 1800 | 0·01 | Killed 269 | M.T.(*d*) |
| Guinea-pig. | 620 | 460 | 10·0 subcut. | Died 20 | E.T. |
| | 640 | 445 | 10·0 | 68 | A.T. |
| | 670 | 565 | 0·1 | 115 | A.T. |

(*a*) Small local lesion and few small surface lesions in lungs.
(*b*) Chronic type, lungs pale and showing large cavities.
(*c*) Just visible dark points in lungs.
(*d*) Condition good; old cavities in both lungs.

## Dog 6.

This animal was a male wire-haired fox-terrier, about three years old. Dullness, wasting, and a chronic cough had been noticed for some months previously.

*Post-mortem Examination.*

The chest wall of the right side showed numerous disc-like firm nodules, in size from $\frac{1}{2}$ mm. to 3 mm.; towards the diaphragm were larger rounded lesions up to 2 cm. diameter, clustered thickly near it. One such lesion was present on the left chest wall. The diaphragm showed a few yellowish nodules, chiefly of button form and up to 2 cm. on its pleural surface and also a few larger masses of lesions which had coalesced. The anterior mediastinum contained a yellowish mass about 3 by 2 cm.; the posterior mediastinal region

was practically one lobulated mass of tuberculous growths attached to the diaphragm. On section they were white and firm, but softened in parts, and from them a small amount of milk-like fluid escaped.

No other lesions were found.

*Bacteriological Examination.*

Bacilli were not numerous in the lesions and were moderately long and slender. A strain was obtained from the mediastinal lesions through a guinea-pig and proved to be of dysgonic type, growing about equally well on plain and glycerine media.

Quantitative tests showed it to be of high virulence for rabbits though a little lower than the average bovine strain. Guinea-pigs died promptly and showed typical caseo-necrotic lesions.

ANIMAL INOCULATIONS.

| *Animal.* | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|
| *Original inoculations.* | | | |
| Guinea-pig. | Antiformined. | Died 17 | E.T. } * |
| | | 30 | E.T. } |
| Rabbit ... | | 186 | Neg. |
| *Quantitative inoculations* with Besredka culture 221 days later. | | | |
| Rabbit ... | 10·0 mg. subcut. | Died 62 | A.T. |
| | 1·0 | 144 | A.T. |
| | 0·1 | 112 | A.T. |
| | 0·01 intrav. | 59 | A.T. |
| Guinea-pig. | 10·0 subcut. | 39 | A.T. |
| | 1·0 | 35 | A.T. |
| | 0·1 | 74 | A.T. |

* Prevailing Epizootic.

## Dog 7.

The animal was a crossbred Spaniel, one year old, which had been admitted to hospital suffering from broncho-pneumonia and showing a temperature of 104·8°. Tuberculosis was suspected and four days later, when the temperature was normal and steady, 1 cc. of tuberculin was injected subcutaneously; a rise of 2·2° to 103·9°, followed by a regular fall, resulted.

*Post-mortem Examination.*

Both lungs were found to be somewhat congested, dark red in colour, and collapsed at the edges. Pleurisy was marked on the right side. The lung showed one ulcerated lesion about 2 cm. diameter, the tissue being of a greyish necrotic appearance, and a little thin turbid greyish fluid could be squeezed from it.

The remainder of the lungs was sprinkled with small firm greyish lesions up to 2 mm. in diameter. The bronchial and mediastinal glands were slightly enlarged.

*Bacteriological Examination.*

Microscopic examination revealed fairly numerous acid-fast bacilli in the tissue and juice of the ulcerated lesion, and one only in a smear from a bronchial gland. The bacilli were long and thin, mostly beaded and many curved, the majority 3-4 $\mu$, but many longer and shorter.

Strains were obtained direct and through a guinea-pig. The initial direct

cultures were obtained on plain egg, glycerine media being negative. In the next generation, however, growth commenced as quickly on glycerine media as on plain and was eventually greater. By the third generation growth was good on all egg media and fairly good on others. This strain nearly approached the eugonic strains of low virulence in its cultural characters, and it would have been difficult to form an opinion of its type with cultures alone.

Virulence for rabbits was high, and it was thought advisable to obtain strains from the inoculated animals. One was grown from the lung of the rabbit which died eighteen days after intravenous inoculation with 0·01 mg., and another from the lung of the rabbit which received 10·0 mg. subcutaneously and died at 83 days. Both strains were similar to the inoculated strain.

Guinea-pigs died in average time and showed advanced caseo-necrotic lesions.

The virus thus appeared to be of bovine type, but was much less dysgonic than the great majority of bovine viruses.

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit ... | | | Fresh. | Died 102 | A.T. |
| Guinea-pig. | | | | 35 | A.T. |
| *Quantitative tests* with Besredka culture 259 and 349 days later. | | | | | |
| Rabbit ... | 1700 | 1200 | 10·0 mg. subcut. | Died 83 | A.T. |
| | 1500 | 1200 | 0·1 | 90 | A.T. |
| | 1900 | 1600 | 1·0 intrav. | 17 | A.T. |
| | 1600 | 1200 | 0·01 | 18 | A.T. |
| Guinea-pig. | 430 | 365 | 10·0 subcut. | 19 | E.T. (*a*) |
| | 545 | 400 | 10·0 | 24 | M.T. (*b*) |
| | 520 | 500 | 10·0 | 77 | A.T. |
| | 540 | 365 | 1·0 | 24 | E.T. (*c*) |
| | 480 | 410 | 10·0 | 52 | A.T. (*d*) |
| | 540 | 450 | 0·1 | 66 | A.T. |
| | 575 | 550 | 0·1 | 76 | A.T. (*e*) |

(*a*) Spleen studded with small discrete grey lesions, 1/600th of body weight. Liver showed slight disease.
(*b*) Spleen 1/300th of body weight ; liver moderate lesions of caseo-necrotic type.
(*c*) Spleen 1/400th of body weight, lesions chiefly discrete but caseation commencing. Liver slightly infected.
(*d*) Spleen 1/50th of body weight, liver 1/10th.
(*e*) Spleen 1/50th body weight, liver 1/6th.

## Dog 8.

The animal was a male Newfoundland in very poor condition. No history was available and tuberculosis had not been suspected previous to death.

*Post-mortem Examination.*

Lesions were widespread. The serous membranes of both thorax and abdomen were extensively affected, the pleura being considerably thickened and in many places showing distinct grape-like lesions. The lungs were riddled with early lesions of a greyish colour surrounded by a zone of congestion and hepatisation, but not softened. The bronchial glands were enlarged,

in two cases up to 2 cm. diameter, and were of the usual advanced type, *i.e.*, slightly fluctuating and containing a small amount of milky fluid, with small flocculi, in a whitish fibrous reticulum. The mesentery was extensively affected; its glands were enlarged and mostly firm, but a few showed more advanced changes, similar to those in the bronchial glands. Both kidneys were increased 50 per cent. in size and studded with whitish raised tubercles up to 8 mm. in diameter and of conical section. The liver showed about half-a-dozen lesions from 3-10 mm., of firm yellowish-white character, and one twice as large, broken down into a caseo-custard like material.

The carcase glands and other organs appeared normal.

*Bacteriological Examination.*

Acid-fast bacilli were very rarely found and were usually small, short, and evenly stained. About a dozen were found in eleven smears from liver, gland, lungs, and serous membrane.

A strain was obtained from the lung and other strains from the mesenteric glands through guinea-pigs. All were definitely dysgonic. Cultures of the virus appeared to be definitely inhibited by the presence of glycerine in the medium. Colonies did not appear until late on glycerine media (2-3 weeks), and then were flatter and often stellate, the amount of growth obtained finally being less than on plain media. No growth was ever obtained on glycerine agar, and only a very occasional minute colony on glycerine potato, whereas plain media, egg, serum, and serum agar were nearly always covered with visible growth after two weeks incubation. When tested quantitatively on rabbits and guinea-pigs its virulence was found to be very high. The Besredka culture used in this case was one of the oldest used (30 days).

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Antiformined gland. | Died 50 | A.T. |
| Rabbit ... | | | | 67 | A.T. |
| | | | Fresh lung. | 76 | A.T. |
| *Quantitative inoculations with Besredka culture 164 days later.* | | | | | |
| Rabbit ... | 2300 | 1500 | 10·0 mg. subcut. | Died 43 | A.T. |
| | 2400 | 1600 | 0·1 | 37 | A.T. |
| | 2000 | 1500 | 1·0 intrav. | 12 | A.T. |
| | 2000 | 1700 | 0·01 | 23 | A.T. |
| Guinea-pig. | 520 | 415 | 10·0 subcut. | 14 | E.T. |
| | 670 | 415 | 10·0 | 17 | M.T. |
| | | | 0·1 intrap. | 17 | A.T. |
| | 590 | 420 | 0·1 subcut. | 44 | A.T. |

Dog 9.

The subject was a crossbred male terrier, three years old, which belonged to a restaurant proprietor and spent part of its life in the public rooms. As it was emaciated and showed clinical signs of tuberculosis on admission to hospital, tuberculin had been applied subcutaneously in a 1 cc. dose; a moderate but otherwise typical reaction was obtained with a rise in temperature of 1·3° F. The animal was chloroformed to death.

*Post-mortem Examination.*

The lungs each showed small greyish areas up to 4 mm. in diameter. The mediastinum was thickened and showed small rounded excrescences. Bronchial and mediastinal glands were enlarged and œdematous but showed no other alteration. There were extensive liver lesions, and the portal glands were enlarged. Both kidneys showed numerous lesions in the cortex, some extending into the medulla. The pancreas was enlarged and a number of small yellow caseating areas were present. Innumerable shot-like tubercles were present in the mesentery, which was also diffusely thickened.

*Bacteriological Examination.*

Microscopical examination showed very rare acid-fast bacilli in glands, liver, and omentum ; smears from the lungs showed a small number. They were long and slender, with a moderate number of beaded forms. Growth was definitely eugonic even in primary direct cultures and was greatly enhanced by the presence of glycerine in the medium ; it was of a deep buff colour, particularly on egg and serum.

Virulence for rabbits was very low by the subcutaneous route and, if one animal be excepted, low by the intravenous route. It will be seen in the table that one rabbit which received 0·01 mg. intravenously died with advanced lesions not readily distinguishable from those caused by an active bovine virus, but as two other animals which received a similar dose of another culture and one which received 1 mg. intravenously showed no lesions or only slight ones, it will probably be correct to assume a special susceptibility of the rabbit which died.

The bacilli from this case thus appear to be of human type.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit ... | | | Fresh. | Killed 155 | Local. |
| Guinea-pig. | | | | Died 12 | |
| *Quantitative tests* 71 and 184 days later with egg culture. | | | | | |
| Rabbit ... | 2000 | 1500 | 10·0 mg. subcut. | Died 142 | S.T. (*a*) |
| | 2150 | 2500 | 10·0 | Killed 186 | Local. |
| | 1850 | 3300 | 1·0 intrav. | 186 | M.T. (*b*) |
| | 2100 | 1400 | 0·01 | Died 68 | A.T. |
| | 2250 | 2600 | 0·01 | Killed 186 | Neg. |
| | 1650 | 2000 | 0·01 | 186 | S.T. (*c*) |
| | 1750 | 2600 | 1·0 subcut. | 340 | S.T. (*d*) |
| | 2000 | 2250 | 0·1 | 340 | Neg. |
| Guinea-pig. | 710 | 540 | 10·0 | Died 46 | A.T. |
| | 450 | 385 | 1·0 | 34 | M.T. |
| | 700 | 535 | 0·1 | 75 | A.T. |

(*a*) Local lesion and small number of lesions in the lungs, scars in kidneys.
(*b*) Lesions of fibrous and chronic type.
(*c*) Very slight lesions in the lungs.
(*d*) Small number of shot-like bodies in the lungs, left anterior lobe adherent to the pleura of chest wall and showed surface lesions.

## Dog 10.

This animal, a crossbred Irish terrier, six years old, had been the constant companion of a girl who suffered from pulmonary tuberculosis with much expectorate and had died two months before.

It had the characteristic facial appearance, with marked atrophy of the muscles, particularly of the temporal region; respiration was abdominal and there was a slight cough.

*Post-mortem Examination.*

There were advanced lesions in the thorax, which was half filled with a thin slightly milky exudate. The bronchial and mediastinal glands were markedly enlarged and in nearly all cases firm; one bronchial gland was, however, softened and of the usual fluctuating type, and from it a little milky juice was collected for inoculation. Lung lesions were very few, firm and greyish, irregular in shape, and up to 4 mm. in diameter.

The liver showed advanced lesions which were raised, yellowish, usually about ½ cm. in diameter, a few up to 1 cm.; the consistence was firm except in two cases where softening had produced a lesion somewhat resembling the softened lymph gland.

Each kidney showed a few small typical raised cortical lesions.

*Bacteriological Examination.*

Microscopical examination showed a few bacilli in scrapings from lung lesions and a very occasional one in gland and mediastinal lesions. Guinea-pigs inoculated with pleural exudate, juice from a softened gland, and emulsified lung and liver material, all became infected and cultures were obtained.

The cultural characters were those of a typically eugonic strain, which grew much better on glycerine, serial cultures showing well marked differences.

Virulence for rabbits was practically nil by the subcutaneous route and very low by the intravenous.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Lung. | Died 14 | Local. |
| | | | | 38 | A.T. |
| | | | Pleural exudate. | 34 | E.T. |
| | | | Liver and gland. | 38 | M.T. |
| | | | Juice bronchial gland. | 82 | A.T. |
| Rabbit ... | | | Lung and pleural exudate. | 284 | Neg. |
| | | | Liver and gland. | Killed 340 | Neg. |
| *Quantitative inoculations* with Besredka culture 192 days later. | | | | | |
| Rabbit ... | 3500 | 2700 | 10·0 mg. subcut. | Died 31 | Local. |
| | 2700 | 1600 | 10·0 | 131 | S.T. (*a*) |
| | 2700 | 2400 | 0·1 | 68 | Local. |
| | 2200 | 2600 | 0·1 | Killed 186 | |
| | 2600 | 1680 | 1·0 intrav. | Died 123 | M.T. (*b*) |
| | 2800 | 3200 | 0·01 | Killed 186 | S.T. (*c*) |
| Guinea-pig. | 530 | 370 | 10·0 subcut. | Died 27 | A.T. |
| | 770 | 560 | 10·0 | 118 | A.T. |
| | 530 | 420 | 0·1 | 108 | A.T. |
| | 570 | 440 | 0·1 | 120 | A.T. |

(*a*) Local lesion and few small chronic lesions in lungs.

(*b*) Chronic type of disease; lungs showed a small number of old lesions from 1 mm. to 1 cm., larger ones with cavities or composed of soft whitish custard-like material; few lesions in kidneys; both testicles tuberculous.

(*c*) Two small sub-pleural lesions in the right lung; both stifles tuberculous.

## Dog 11.

The animal, an adult mongrel terrier, was very emaciated ; no history could be obtained.

*Post-mortem Examination.*

Advanced lesions were present in the lungs and bronchial glands, but careful search revealed no other lesions. Both lungs were sprinkled with lesions ; the right showed a distinctly larger number, those in the right apical lobe being nearly confluent. They varied from ½ cm. to 2 cm., were irregularly rounded, and of a greyish-red colour. Two of the larger ones showed a central softened point, and, when sectioned, an irregular central cavity covered with a dark greyish-brown exudate in which acid-fast organisms could be easily demonstrated. The bacilli were thin and granular and generally long, but varied from 2-5 $\mu$.

*Bacteriological Examination.*

Direct strains were obtained on plain media and these grew moderately well from the first generation. In subsequent generations growth commenced as quickly (10-14 days) on glycerine media as on plain media, and was eventually distinctly greater. Glycerine potato showed a rather thin whitish growth all over, with raised pearly islands. Small growth of a thin and rather warty character was obtained on glycerine broth, but sank after about a month, when the inoculum had increased to about five times its original size. Growth, however, never approached that given by the human strains.

This strain possessed high virulence for rabbits and guinea-pigs.

Animal Inoculations.

With serum cultures 95 days after the original material was received.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| Rabbit ... | 1875 | 1000 | 10·0 mg. subcut. | Died 70 | A.T. |
| | 1900 | 1720 | 0·1 | 105 | A.T. |
| | 1800 | 1500 | 1·0 intrav. | 24 | A.T. |
| | 2400 | 2000 | 0·01 | 35 | A.T. |
| Guinea-pig. | 570 | 440 | 10·0 subcut. | 24 | E.T. |
| | 370 | 355 | 10·0 | 37 | A.T. |
| | 395 | 345 | 0·1 | 61 | A.T. |
| | 385 | 375 | 0·1 | 80 | A.T. |

## Dog 12.

The animal was a Manchester terrier, about two years old, and had belonged to a man who was admitted to hospital twelve months before, suffering from pulmonary tuberculosis ; he had since returned home in better health.

The animal had a characteristic appearance and was slightly lame, the bones of all legs being enlarged, particularly in the region of the joints.

Ophthalmic and intradermal tuberculin tests were negative. The subcutaneous test was applied on two successive days and gave distinctly positive indications, but the pre-inoculation temperature was so variable as to make difficult the evaluation of the moderate rise of 1·5° F.

*Post-mortem Examination.*

Each of the lobes of both lungs showed on the surface one or more rounded slightly raised areas, from 1 cm.-2 cm. diameter, and in addition a few smaller

lesions. The surface was of a greyish-red colour and somewhat more shiny than usual. In three of the larger lesions pressure showed the presence of a central cavity, which was of the usual type and contained a small amount of fluid. The other lesions were composed of a greyish rather soft type of tissue, showing in most cases tiny specks of greenish-white turbid material, with definite small pockets in a few of the larger ones.

*Bacteriological Examination.*

Microscopical examination showed acid-fast bacilli in small numbers both in the material from the cavities and in scrapings from the hepatised and necrotic lung. These were present generally in small clumps up to a dozen individuals, and were long, curved, and granular, 3-4 $\mu$ in length, many longer.

Cultures were obtained direct, and in the second and subsequent generations were found to be typically eugonic, growing quickly and profusely on all media. Growth on glycerine broth was obtained at the second generation and at the next transfer quickly covered the surface with a whitish wrinkled growth.

Virulence for the rabbit was low, but the animal which received 1 mg. intravenously died after twenty-two days and showed advanced lesions indistinguishable from those caused by a virulent bovine virus. In spite of this result, the strain must be classed as of human type on account of its cultural characters and the absence of any but slight lesions in rabbits inoculated subcutaneously and in those given 0·01 mg. intravenously.

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Orig. mat. subcut. | Died 16 | Local. |
| | | | intrap. | 24 | A.T. |
| Rabbit ... | | | subcut. | 320 | S.T. (*a*) |
| *Quantitative inoculations with serum culture 103 days later.* | | | | | |
| Rabbit ... | 1700 | 2100 | 10·0 mgm. subcut. | Died 215 | S.T. (*b*) |
| | 2400 | 2300 | 0·1 | 186 | Neg. |
| | 2200 | 1300 | 1·0 intrav. | 22 | A.T. |
| | 1800 | 1100 | 0·01 | 196 | S.T. (*c*) |
| | 2100 | 2500 | 0·01 | Killed 210 | Neg. |
| Guinea-pig. | 380 | 295 | 10·0 subcut. | Died 23 | M.T. (*d*) |
| | 415 | 335 | 0·1 | 23 | M.T. (*e*) |
| | 395 | 520 | 0·1 | 94 | A.T. (*f*) |
| | 345 | 275 | 0·1 | 106 | A.T. (*g*) |

(*a*) Small lesions in lungs and testicles.
(*b*) Large local lesion and a few small lesions in lungs.
(*c*) Shot-like lesions in lungs only.
(*d*) Spleen 1/260th of body weight. Liver 1/20th.
(*e*) Spleen 1/300th of body weight. Liver 1/20th.
(*f*) Lesions not of caseo-necrotic type.
(*g*) Spleen 1/35th of body weight and lesions of hæmorrhagic type.

## Dog 13.

The animal was a crossbred Samoyede dog, aged nine years, and was the property of a family the mother of which had died two years previously from phthisis.

For several weeks before admission to hospital it had been losing condition,

and on examination it was found to be exceedingly emaciated and weak ; the temporal muscles were wasted, and eyes overbright and sunken.

An indefinite but slight positive response had been obtained to tuberculin subcutaneously.

*Post-mortem Examination.*

Each kidney showed about six tuberculous lesions, whitish, distinctly raised, and about 2 mm. in diameter. The liver was distinctly enlarged and covered with miliary whitish tubercles, $\frac{1}{2}$-2 mm. in diameter. The pleura, diaphragmatic and costal, was covered with nearly flat papilliform lesions about 2 mm. in diameter ; a few were of more irregular type and up to 1 cm. in diameter.

The lungs were riddled with firm greyish lesions from 1 mm. up to $1\frac{1}{2}$ cm., very irregular in appearance and distribution. On the left side the diaphragmatic lobe was completely involved, twice the normal size, and fibrous, and the cardiac lobe was severely involved and adherent to the bronchial glands, which were markedly enlarged and had the usual characters of a softened tuberculous gland of the dog. The remaining left lobe and the right lung showed no large lesion. No cavities were present. The mass formed by the bronchial glands of both sides was about 5 cm. in diameter. Section showed the usual whitish fibrous reticulum containing a small amount of milky fluid with small flocculi.

A supra-sternal gland was much enlarged, greyish-white and not softened. Considerable bone changes were present, particularly in the joints of the left hind legs below the stifle.

Animal Inoculations.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Orig. mat. | Died 16 | Local. |
| | 345 | 260 | | 21 | S.T. |
| | 770 | 650 | intrap. | 21 | E.T. |
| Rabbit ... | 1800 | 1620 | | Killed 151 | Neg. |
| *Quantitative inoculations with serum cultures 167 days later.* | | | | | |
| Rabbit ... | 2150 | 2400 | 10·0 mg. subcut. | Killed 111 | S.T. (*a*) |
| | 1920 | 1180 | 0·1 | Died 33 | Local. |
| | 1750 | 2400 | 0·1 | Killed 111 | |
| | 2150 | 1800 | 1·0 intrav. | 111 | M.T. (*b*) |
| | 2000 | 2150 | 0·01 | Died 96 | S.T. (*c*) |
| Guinea-pig. | 630 | 510 | 10·0 subcut. | 16 | M.T. |
| | 425 | 310 | 10·0 | 52 | A.T. |
| | 605 | 560 | 0·1 | 101 | A.T. (*d*) |
| | 605 | 450 | 0·1 | 101 | A.T. (*e*) |

(*a*) Local lesion and few shot-like lesions in lungs.
(*b*) Lesions of chronic type in lungs, kidneys, and testicles.
(*c*) Tiny glistening bluish lesions in lungs.
(*d*) Liver 1/9th body weight, not caseo-necrotic. Spleen 1/35th of body weight, partly hæmorrhagic, partly caseated.
(*e*) Liver 1/10th body weight and cirrhotic. Spleen 1/30th body weight and caseo-necrotic.

*Bacteriological Examination.*

Acid-fast bacilli were very rare, only six being found in four smears from lung and three from glands. The bacilli seen were all over 3 $\mu$ and definitely granular.

The strain was obtained from a guinea-pig inoculated with mixed gland and lung material.

It soon became apparent from differential sowings that this strain was definitely eugonic and favoured by glycerine ; nevertheless, good growths were quickly obtained on all media.

Virulence for rabbits was low ; the lesions and marked loss of weight found in the animal which received 1 mg. intravenously were such as might have been expected as a result of this large dose.

### Dog 14.

The animal was a four years old male Airedale ; no history was available as it had been in the owners possession only six months. Emaciation was marked and the temporal muscles were distinctly wasted ; definite enlargement of the throat glands could be detected clinically and one of these was connected with the surface by a fistula, which was discharging. Polyuria had been noticed. Auscultation had revealed marked râles over the left lung.

Twenty minims of tuberculin had been given subcutaneously and had given rise to a characteristic reaction with a rise in temperature of 2° F.

*Post-mortem Examination.*

The anterior part of the mesentery showed very small tubercles. The lymphatic glands were slightly enlarged and contained small tubercles. The liver showed about 200 lesions, scattered fairly evenly, varying from 2 mm. to 1 cm. in diameter and with a large yellowish necrotic central area.

Each kidney showed one or two tubercles, smaller and less raised than is commonly the case.

The abdominal surface of the diaphragm was covered with a diffuse yellowish deposit ; the peritoneal fluid was little increased or altered.

The lungs were moderately affected, with lesions which were distributed fairly evenly throughout ; they varied from a few mm. up to 1 cm. in diameter and were of a purplish-grey colour with a lighter centre, which was generally slightly raised and in some cases soft. The latter, when cut open, showed a central irregular cavity of the usual type, containing a small amount of greyish turbid liquid.

The pleura was normal and there was no excess of fluid.

The lymph glands in the region of the throat were much enlarged and in the retropharyngeal group were pressing on the back of the pharynx. Section showed them to be composed of a whitish fibrous reticulum from which could be pressed a small amount of milky slightly fatty-looking liquid. Palpation gave the impression of a thin capsule containing a firm rubber sponge. The gland connected with the fistula showed areas of thick yellowish pus, probably caused by secondary organisms. The infection appeared to have commenced in the pharyngeal glands.

*Bacteriological Examination.*

Microscopic examination revealed a few acid-fast bacilli in material from each of three lung lesions. No bacilli were found in any other lesions or in lungs or liver. The red-stained (Ziehl-Neelsen) bacilli showed in many cases very intensely stained blackish granules, and were long and often curved or S-shaped, length 3-6 $\mu$ (average of twenty individuals).

Cultures were obtained direct from the lung material and through a guinea-pig from the liver. They appeared similar.

This strain did not grow well in the first few generations, but gradually

improved and from the fourth generation was typically eugonic. Its preference for glycerine media was particularly well brought out in serial cultures.

Virulence for rabbits was low, both subcutaneously and intravenously. Guinea-pigs survived a long time, and those which received the lower dose showed at death large lesions in which caseation was not a marked feature; they were of the type commonly associated with inoculation of a strain of low virulence for rabbits.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit ... | | | Orig. mat. | Died 10 | Neg. |
| Guinea-pig. | 515 | 620 | | 28 | E.T. |
| | | | | 27 | E.T. |
| *Quantitative inoculations with serum cultures 148 days later.* | | | | | |
| Rabbit ... | 2590 | 2100 | 10·0 mg. subcut. | Died 52 | S.T. (*a*) |
| | 2200 | 2600 | 0·1 | Killed 111 | Local. |
| | 1900 | 2240 | 1·0 intrav. | 111 | S.T. (*b*) |
| | 1700 | 1800 | 0·01 | 111 | S.T. (*c*) |
| Guinea-pig. | 320 | 380 | 10·0 subcut. | Died 58 | A.T. |
| | 590 | 440 | 10·0 | 73 | A.T. |
| | 680 | 580 | 0·1 | Killed 111 | A.T. (*d*) |
| | 420 | 585 | 0·1 | 111 | A.T. (*e*) |

(*a*) Local lesion, lungs crepitant and showed numerous just visible greyish lesions; two in the right lung were 2 mm. and had tiny caseated centres. Cause of death—coccidiosis.
(*b*) Few small lesions in lungs only.
(*c*) Few tiny glistening lesions in lungs.
(*d*) Spleen 1/60th body weight; liver 1/10th and of cirrhotic type, no caseation.
(*e*) Spleen 1/40th body weight and not markedly caseo-necrotic. Liver 1/16th body weight and cirrhotic type with no caseation.

## DOG 15.

This animal, a crossbred male terrier, aged six years, belonged to a man who suffered from pulmonary tuberculosis; the dog was known to ingest the expectorate. It was emaciated and had a cough; temperature ranging from 101·4° to 102° F.

Twenty minims of tuberculin had been injected subcutaneously and produced a typical reaction with a rise of 2° to 103·6° F. and steady fall.

*Post-mortem Examination.*

The bronchial and mediastinal glands were fused to form a rounded mass about 8 cm. diameter, which was seen on section to be of the usual reticular type, from which a small amount of milky fluid could be expressed. A few smaller lesions were present in the anterior mediastinal region, and one rounded mass, about 1½ cm. in its longest diameter, was attached to the exterior of the pericardium; there was also one small greyish papillomatous lesion attached to the left costal pleura.

The lungs and other organs were unaffected.

*Bacteriological Examination.*

Microscopic examination showed no acid-fast bacilli, but cultures were obtained on broth egg and glycerine broth egg and when transferred to differential media soon gave evidence that they were definitely eugonic and favoured by glycerine ; growth was always a little slow.

Low virulence for rabbits agreed in showing that this strain was of human type.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Fresh. | Died 52 | A.T. |
| | | | | 102 | A.T. |
| *Quantitative inoculations with serum culture 93 days later.* | | | | | |
| Rabbit ... | 2020 | 2270 | 10·0 mg. subcut. | Killed 97 | S.T. (*a*) |
| | 2240 | 2710 | 0·1 | 97 | Local. |
| | 2270 | 2210 | 1·0 intrav. | Died 13 | S.T. (*b*) |
| | 2500 | 1740 | 1·0 | 21 | S.T. (*c*) |
| | 2120 | 2300 | 1·0 | Killed 97 | M.T.(*d*) |
| | 2180 | 2500 | 0·01 | 97 | S.T. (*e*) |
| Guinea-pig. | 565 | 430 | 10·0 subcut. | Died 54 | A.T. |
| | 580 | 425 | 10·0 | 42 | A.T. |
| | 640 | 650 | 0·1 | 124 | A.T. |
| | 460 | 350 | 0·1 | 65 | A.T. |

(*a*) Local ; a few irregular lesions up to 4 mm. with caseated centres and a few glistening shot-like lesions. Kidneys show each one or two small regressive lesions.

(*b*) Multiple pin-point lesions in lungs ; no bacilli in blood.

(*c*) Lungs crepitant but riddled with tiny greyish lesions. Apex of anterior lobe of right lung and edges of other lobes solid.

(*d*) Both lungs crepitant but showed a moderate number of chronic lesions 2-6 cm. and numerous shot-like glistening lesions.

(*e*) Few small lesions in lungs, up to 1 cm., but majority shot-like.

## DOG 16.

This animal, a crossbred black terrier, six years old, was markedly emaciated; it came from a private house which had no history of tuberculosis.

*Post-mortem Examination.*

There was a small lesion of the diaphragmatic pleura and one caseous thoracic gland about $\frac{1}{2}$ cm. diameter. The lungs showed small hæmorrhagic foci only. The liver was extensively involved, numerous small discrete lesions being present throughout ; in the right lobe were many larger lesions up to 1 cm. diameter and of usual type, and in the left lobe one softened lesion about 4 cm. diameter, from which on section a moderate amount of milky fluid escaped. The stomach was adherent at this part. The mesentery was slightly involved and thickened. A small tubercle was present in the cortex of one kidney.

*Bacteriological Examination.*

Microscopic examination revealed no acid-fast bacilli and direct cultures failed, but a strain was obtained through a guinea-pig which died after 89 days, showing advanced tuberculosis.

In cultural characters this strain was typically eugonic, growth being rapid and profuse on all media and particularly on those containing glycerine.

Animal inoculations showed it to possess the virulence of a typically human strain.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | |
| *Original inoculations.* | | | | | |
| Rabbit ... | | | Fresh. | Killed 182 | Neg. |
| Guinea-pig. | | | | Died 89 | A.T. |
| | | | | 108 | A.T. |
| *Quantitative inoculations* with serum culture 133 days later. | | | | | |
| Rabbit ... | 2100 | 2900 | 10·0 mg. subcut. | Killed 111 | S.T. (*a*) |
| | 2000 | 2000 | 0·1 | 111 | S.T. (*b*) |
| | 2300 | 2200 | 1·0 intrav. | 111 | M.T. (*c*) |
| | 2150 | 2150 | 0·01 | 111 | S.T. (*d*) |
| Guinea-pig. | 555 | 450 | 10·0 subcut. | Died 52 | A.T. |
| | 425 | 270 | 10·0 | 40 | A.T. |
| | 590 | 670 | 0·1 | 111 | A.T. (*e*) |
| | 470 | 730 | 0·1 | Killed 111 | A.T. (*f*) |

(*a*) Local lesion and very slight lesions in the lungs.
(*b*) Small local lesion and very slight lesions in lungs.
(*c*) Large white lesions with central cavities with softened material. Right kidney one lesion extending into medulla ; both a few scars.
(*d*) Fairly numerous small lesions in lungs, many shot-like.
(*e*) Liver 1/12th body weight ; spleen 1/70th.
(*f*) Liver 1/20th body weight ; spleen 1/150th.

## PARROT.

The bird had shown a small swelling of the lower eyelid for some time. The lesion was found to be of a caseous nature and microscopic examination of its contents showed it to contain numerous acid-fast bacilli. A strain was obtained through a guinea-pig .

*Bacteriological Examination.*

Culturally it was definitely of eugonic character and not avian-like, quickly producing a good growth on most media and particularly on those containing glycerine. On glycerine agar and potato, however, it developed a little slower and never produced quite as good a growth as most strains of human type.

The first animal experiments (not reported) were badly interfered with by intercurrent disease amongst the guinea-pigs, but showed that this virus was of low virulence for rabbits. The second series inoculated showed that it was of low virulence for the rabbit and avirulent for fowls, but in this series three of the rabbits were killed by other diseases. A third series showed definitely that whilst virulent for guinea-pigs it was of very low virulence for the rabbit. The results of the inoculations with the original material were in accordance with these results.

The strain thus appeared to be of human type.

It has since been found that this bird was the pet of a man who suffered from phthisis and that human infection had been suspected.

ANIMAL INOCULATIONS.

| Animal. | Weight in grammes. Initial. | Final. | Material and Dose. | Survival in Days. | Result. |
|---|---|---|---|---|---|
| *Original inoculations.* | | | | | |
| Guinea-pig. | | | Fresh material. | Died 53 | A.T. |
| Rabbit ... | | | | Killed 160 | Local. |
| *Quantitative inoculations* with Besredka culture 610 days later. | | | | | |
| Guinea-pig. | 560 | 460 | 10·0 mg. subcut. | Died 91 | A.T. |
| | 670 | 410 | 10·0 | 59 | A.T. |
| | 790 | 605 | 0·1 | 83 | A.T. |
| | 640 | 600 | 0·1 | 91 | M.T. |
| Rabbit ... | 2500 | 1250 | 10·0 | 98 | S.T. (*a*) |
| | 2600 | 2000 | 1·0 intrav. | 84 | Neg. (*b*) |
| | 2000 | 1900 | 0·01 | 96 | S.T. |
| Fowl ... | | | 1·0 intrmuscu'r | Killed 175 | Neg. |
| | | | 1·0 | 175 | Neg. |
| | | | 1·0 intrav. | Died 41 | ? Neg. (*c*) |
| | | | 0·01 | Killed 175 | Neg. |
| *Quantitative inoculations* with serum culture 700 days later. | | | | | |
| Rabbit ... | 2000 | 3500 | 0·1 mg. subcut. | Killed 158 | Local. |
| | 2300 | 2850 | 1·0 intrav. | | S.T. (*d*) |
| | 1700 | 2600 | 1·0 | | S.T. (*e*) |
| | 2000 | 2850 | 0·01 | | Neg. |
| | 2300 | 3200 | 0·01 | | S.T. (*f*) |

(*a*) Coccidiosis. Local and small lesions in lungs, which were crepitant.
(*b*) Pneumonia, but unlike tuberculosis, and no acid-fasts found after prolonged search.
(*c*) A few small greyish lesions in lungs only, but no tubercle bacilli in smears from them, spleen, or blood.
(*d*) A few small greyish lesions in lungs, testicles, and sacculus rotundus.
(*e*) Lungs each showed 20-30 lesions 2-4 mm. in diameter, greyish and of chronic type.
(*f*) A few tiny greyish subpleural lesions.

## A WALLABY.

This animal had been kept in a small paddock together with birds (chiefly fowls), rabbits, and other small rodents.

*Post-mortem Examination.*

The abdominal organs only were received; the thorax was said to be normal.

The liver was riddled with yellowish tubercles, generally rounded, and in size from ½ mm. to 1 mm. with occasional lesions up to 3 mm. On section they were firm and not easily broken up. The portal gland was 4 by 3 by 2 cm., and completely caseated, with some evidence of calcification. The spleen was beset with lesions similar to those in the liver, and in many places they had coalesced to form large confluent masses. Each kidney showed superficially and deep in the cortex a few tubercles up to 3 mm. in diameter. One mesenteric gland was enlarged to 6 by 4 by 3 cm., caseated to an advanced degree, and slightly calcified; a few glands were affected to a less degree.

ANIMAL INOCULATIONS.

| *Animal.* | *Weight in grammes.* | | *Material and Dose.* | | *Survival in Days.* | *Result.* |
|---|---|---|---|---|---|---|
| | *Initial.* | *Final.* | | | | |
| Guinea-pig. | | | Fresh hepatic gland. | | Killed 190 | Local (*a*) |
| Rabbit ... | | | | | 190 | S.T. (*b*) |
| *Quantitative tests* with glycerine agar culture 307 days later. | | | | | | |
| Rabbit ... | 1900 | 1750 | 10·0 mg. | subcut. | Died 62 | M.T. |
| | 2000 | 1600 | 1·0 | | 116 | M.T. (*c*) |
| | 1240 | 1260 | 0·1 | | 57 | M.T. (*d*) |
| | 1800 | 1360 | 0·01 | intrav. | 24 | T. (*e*) |
| | 2000 | 1360 | 0·01 | | 18 | T. (*f*) |
| | 2150 | 1400 | 0·01 | | 77 | A.T. (*g*) |
| Guinea-pig. | 530 | 980 | 10·0 | subcut. | Killed 300 | Local. |
| | 540 | 890 | 1·0 | | 300 | Neg. |
| | 570 | 370 | 0·1 | | Died 57 | Local. |
| Fowl ... | | | 0·01 | intrav. | 33 | T. (*h*) |
| | | | 1·0 | intramus'r. | 62 | T. (*i*) |

(*a*) Condition good, small local lesion, and one in the popliteal gland about 2 mm. diameter.
(*b*) Cystic local lesion at back of femur, iliac glands 2 mm. and 4 mm. in longest diameter respectively, and cystic with necrotic walls. Sacculus rotundus affected.
(*c*) Lungs crepitant and slightly infected, cystic local lesion, iliacs partly calcified, slight lesions in kidney and spleen.
(*d*) Lungs crepitant and sprinkled with hard 1 mm. tubercles, lesions very widespread in glands and organs, but of moderate character.
(*e*) Spleen teeming with tubercle bacilli and slightly enlarged; no other lesions.
(*f*) Complicated with pneumonia and pericarditis due to a small Gram-negative bacillus. Spleen slightly enlarged and on section shewed clumps of tubercle bacilli, surrounded by distinct microscopic changes; no bacilli in Malphigian bodies.
(*g*) See text.
(*h*) Spleen enlarged 2-3 times, bacilli very numerous. Lungs showed a small number of partly caseated lesions, particularly at the edges.
(*i*) Breast nearly half necrotic and firm; spleen showed ½ mm. firm lesions; liver small points.

*Bacteriological Examination.*

Numerous acid-fast bacilli were demonstrated in each type of lesion; the majority were short, but few very long filaments were seen. A strain was obtained direct and also one from a rabbit inoculated with material from the hepatic gland. The cultural characters of the two strains were identical. Growth was of a moist, unctous, and easily emulsified character, separate colonies being almost white, shining, and hemispherical; after a few weeks incubation small secondary colonies sometimes developed and in these microscopic examination showed occasional branched forms. In the primary cultures from the rabbit the growth of colonies actually on the fragments of inoculated tissue was most distinct. This phenomenon has been noted by A. S. Griffith as a characteristic of avian viruses and has been confirmed in three of my own cases—two fowls and one pheasant. Development was good on all media and particularly on glycerine agar and other media containing glycerine. Both strains appeared to have the characters of a typical avian virus.

Rabbits, guinea-pigs, and fowls were inoculated with the direct strain. In two guinea-pigs a small local lesion and lesions in the nearest gland were produced, and in the other no lesion. A moderate degree of virulence was

shown for rabbits subcutaneously, widespread carcase lesions being produced whilst lung infection was slight in comparison. The results obtained by the intravenous inoculation of 0·01 mg. into two rabbits were interesting in that the bacilli seemed to have multiplied to a marked degree without producing any other lesion but a small degree of enlargement of the spleen ; sections of the latter were literally teeming with tubercle bacilli, in one case, in singles and two and threes, and in the second, which was affected with other disease, in groups up to 100, each surrounded by a definite zone of degeneration. A third rabbit—(*g*) in the table—which received a similar dose, lived 77 days and then showed definite lesions in most organs, but of a character quite different from that of the typical bovine virus. The spleen was markedly enlarged and showed very definite lesions, whereas the lungs were closely beset with 1 mm. whitish lesions, with a tiny greyish centre ; the tissue surrounding these lesions appeared to be functioning normally.

Virulence for the fowl was also high.

## Summary.

*Wallaby.*

One case due to bacilli of the avian type.

*Parrot.*

One case due to bacilli of the human type.

*Five Cases in the Cat.*

In one animal lesions were confined to the thoracic cavity ; in the other four infection appeared to have occurred by ingestion.

The bacilli in four cases were dysgonic and of standard bovine virulence. In the other case the bacilli, although typically bovine in their dysgonic cultural characteristics, appeared to be of distinctly lower virulence than standard bovine bacilli.

These facts are in agreement with the evidence that the cat, although susceptible to artificial infection with bovine tubercle bacilli, is very resistant to the other types of tubercle bacillus.

*Sixteen Cases in the Dog.*

In ten cases the thoracic organs were either the only ones affected, or appeared, from the extent and character of the lesions present, to be the place of primary infection. In one case a mesenteric gland presented the only lesion, and in another the pharyngeal region showed the oldest and most extensive lesions. The remaining four cases showed thoracic and abdominal lesions of such a nature that there was considerable doubt as to the earliest changes.

Bacilli with the eugonic cultural characteristics and low virulence of the human type of tubercle bacillus were isolated from each of ten cases ; from the remaining six cases were obtained strains which, in respect of cultural characters and virulence, were of standard bovine type.

That the bacilli with the characters of human type were actually from a human source is supported by the fact that although experiments have been carried out in an endeavour to modify the bovine bacillus by passage through the dog no modification has been produced.

There is sufficient evidence in existence to show that the dog, although resistant to infection by all tubercle bacilli, can be artificially infected with bacilli of both mammalian types. The proportion of natural cases caused by each variety would seem to depend merely on the particular kind of bacillus to which the animal has been exposed.

An endeavour was made to ascertain the particular environment in which each animal had lived, but was only partially successful owing to the reluctance of many owners to give information.

Of six cases due to human bacilli in which useful evidence was obtainable, definite association with a case of human tuberculosis was present in four cases, and apparently absent in two. In the remaining cases no history of human tuberculosis or no history at all was obtained.

The resistance of the dog to the tubercle bacillus is well shown by the character of the lesions, which nearly always suggest a very slow development both in the cases of primary lesions and in those which have followed the transport of a small number of bacilli by blood or lymph. An acute miliary tuberculosis appears to be rare, and even when the lungs present such a number of more or less evenly distributed tubercles as to suggest a massive blood infection the lesions are usually very firm, almost translucent and shot-like in appearance, and the surrounding tissues do not show, macroscopically or microscopically, that acute degree of reaction seen in miliary tuberculosis of the larger domestic animals. The production of massive caseated lesions is also conspicuous by its absence ; primary lung lesions generally become fibrotic, wholly or in part, or gradually break down into a greyish pus-like material, which is absorbed or expectorated, so leaving an irregular cavity which may be walled by tissue of a fibrous nature or by lung tissue in a catarrhal state.

The lesions which occur in other organs nearly always show a similar absence of acute phenomena ; that is well exemplified in the hepatic, pleural, and glandular lesions.

It is only in the lung that tubercle bacilli are commonly present in more than a small number ; there, tissue in the early catarrhal stage usually shows a moderate number of bacilli, as does also the small amount of liquid material which can be obtained from a cavity, but in other lesions the bacilli are notably scarce. It is rare to find more than a very small number of bacilli in other organs, and long search may fail to reveal any in glandular lesions or in the type of new tissue generally associated with mediastinal infection. The morphological characters of the bacilli are extremely variable and give no useful guide as to the type of the infecting bacillus.

Nearly all the dogs and cats affected with tuberculosis came from the clinic of the Royal Veterinary College, and I wish to express my indebtedness to Professor Smythe, with whom the post-mortem examinations have been made or from whom suitable material has been received. All the details of clinical symptoms and tuberculin tests were supplied by him.

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# THE MERCURIC CHLORIDE TEST FOR TRYPANOSOMIASIS IN CAMELS. MECHANISM OF THE REACTION.

By

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In a recent paper by Bennett and Kenny[1] on the serological diagnosis of trypanosomiasis in camels, the advantages of using mercuric chloride as a diagnostic agent have been emphasised. Their paper suggests that the serum changes that take place in infected animals are of a quantitative nature and that by using a dilution of the salt ($HgCl_2$) in distilled water of 1-20,000 infected can be distinguished from normal sera. At this dilution normal sera are uniformly negative while sera of trypanosomiasis cases give reactions varying from slight

haziness to a marked precipitate. Further, heavily infected sera give an opacity even with distilled water, and the suggestion is made that the " whole phenomenon is referable to the globulin fractions of the serum."

In that paper reference was also made to the earlier development of the mercuric chloride test as compared with the formol-gel reaction.

As several infected camels were available, it was decided to carry out preliminary investigations of the serum fractions parallel with mercuric chloride tests. Seeing that the infected individuals were random cases collected from the field, little more could be done than to compare their sera with those of a few normal camels, especially as no information was available as to the proportions of the various fractions in camels of any kind.

Table I gives the results of these preliminary estimations (the technique will be detailed later).

TABLE I.

ESTIMATIONS OF TOTAL PROTEIN AND FRACTIONS IN INFECTED AND NORMAL CAMELS RELATIVE TO THE MERCURIC CHLORIDE REACTIONS, IN GRAMMES PER 100 C.C. OF SERUM.

| *Camel No.* | *Mercuric chloride* 1-20,000 | *Total Protein* | *Albumin* | *Pseudo-Globulin* | *Eu-Globulin* | *Remarks* |
|---|---|---|---|---|---|---|
| 43 | — | 6·39 | 3·71 | 1·53 | 1·15 | Normal |
| 44 | — | 6·41 | 4·01 | 1·26 | 1·14 | ,, |
| 88 | ± | 5·91 | 3·65 | 1·14 | 1·12 | Old infected case treated with Naganol |
| 158 | + | 6·02 | 2·88 | 1·68 | 1·46 | Infected |
| 6 | ++ | 6·62 | 2·97 | 1·05 | 2·60 | ,, |
| 35 | ++ | 6·03 | 3·02 | 0·65 | 2·36 | ,, |
| 81 | + | 5·78 | 2·49 | 1·28 | 2·01 | ,, |
| 173 | + | 6·89 | 2·50 | 2·03 | 2·36 | ,, |
| 5 | ++ | 5·77 | 2·20 | 1·12 | 2·45 | ,, |
| 67 | + | 5·74 | 2·25 | 1·14 | 2·35 | ,, |
| 32 | + | 5·40 | 2·20 | 1·00 | 2·20 | ,, |
| 40 | ++ | 6·01 | 2·55 | 0·82 | 2·64 | ,, |

The figures in Table I show that in the normal camels the quantities of pseudo-globulin were greater than those of eu-globulin, while in the infected camels the reverse was the case.

Moreover, the individuals in which the eu-globulin excess was greatest are among those giving the stronger reactions to mercuric chloride.

In brief, these findings gave sufficient support to the suggestion that globulins were the essential substances concerned in the reaction to warrant more methodical investigation.

The aim of the present work was to follow the development of both formol-gel and mercuric chloride tests in experimentally infected camels. It was also hoped after a chronic infection (as indicated by a steady titre of these tests) had been reached to cure the animals with " Bayer 205," " Naganol," and to follow the subsequent serological changes.

Unfortunately, owing to hyperacute infections with sudden death, it has been possible to carry out the latter with only one animal.

The animals selected were healthy camels negative to the mercuric chloride and formol-gel tests and proved free from infection by repeated blood examinations and by inoculating the blood into rats. They were fed on the usual stock diet and were not worked. Before infecting them repeated mercuric-chloride and formol-gel tests and protein estimations were made to establish normal figures.

### *Technique.*

The mercuric chloride reaction was carried out as previously recommended[1] *viz.*, 1 drop of serum to 1 c.c. of the dilution of the salt in distilled water. In the water test distilled water was substituted for the salt. The symbols ±, +, ++, +++, etc., express the various changes from mere haziness to a marked precipitate.

The proteins were estimated by a slight modification of Wu's colorimetric method.

The terms eu- and pseudo-globulins refer to those fractions of the serum which are precipitated by one-third and one-half saturation with ammonium sulphate, respectively, without further purification.

The readings, both of tests and estimations in the case of each individual, are herewith set out in tabular form.

## Discussion.

Before infection the mercuric chloride test was negative in all cases at a dilution of 1-20,000, while the 1-15,000 dilution in some cases gave slight positives, thus confirming the previous selection of the former dilution as the standard.

The protein figures showed the albumin as constituting rather over 50 per cent. of the total protein, the ratio of eu-globulin to pseudo-globulin being 0·77. The total serum proteins remained fairly constant at about 6 gm. per 100 c.c. serum.

As far as we are aware, no previous figures have been published of the normal protein fractions of camels ; these estimations, however, show a general similarity to Hartley's figures for the proteins of cows, but with less fluctuation.

After infection the mercuric chloride reaction became positive to the lowest titre on the average in 13 days, with extreme limits of nine and 18, and during the succeeding weeks the titre rose rapidly until positive results were given by water alone. The proteins showed no significant changes for some weeks ; later a slight increase became apparent in the eu-globulin fraction, and this fraction continued to increase until, at the height of the infection, the normal eu- : pseudo-globulin ratio was altered from 0·77 to 1·6 or 1·7. The increase of eu-globulin was chiefly at the expense of the pseudo-globulin, the albumin remaining approximately constant.

In a few cases, the total proteins appeared to undergo a small diminution, which was probably of no significance.

CAMEL No. 15.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumin | Pseudo-Globulin | Eu-Globulin | Microscopic examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 20.10.28 | — | — | — | — | — | — | 6·16 | 3·46 | 1·51 | 1·22 | — |
| 27.10.28 | — | — | — | — | — | — | 0·07 | 3·30 | 1·51 | 1·26 | — |
| 28.10.28 | | | | | Given *Trypanosoma soudanense* | | | | | | |
| 6.11.28 | — | — | — | — | — | — | 6·11 | 3·35 | 1·52 | 1·24 | — |
| 15.11.28 | + | + | ± | — | — | — | 6.16 | 3·40 | 1·50 | 1·26 | + (rare) |
| 22.11.28 | + | + | + | ± | — | — | 6·19 | 3·45 | 1·48 | 1·26 | — |
| 27.11.28 | ++ | ++ | ++ | + | — | — | 6·12 | 3·45 | 1·45 | 1·22 | — |
| 3.12.28 | ++ | ++ | ++ | + | — | + | 6·20 | 3·57 | 1·43 | 1·20 | — |
| 10.12.28 | ++ | ++ | ++ | + | ± | + | 6·30 | 3·68 | 1·26 | 1·36 | — |
| 17.12.28 | ++ | ++ | ++ | + | ± | ± | 6·17 | 3·08 | 1·18 | 1·91 | — |
| 24.12.28 | ++ | ++ | ++ | + | ± | ± | 6·04 | 3·24 | 1·12 | 1·68 | — |
| 31.12.28 | ++ | ++ | + | ± | ± | — | 5·76 | 2·91 | 1·12 | 1·73 | — |
| 7.1.29 | ++ | ++ | ± | ± | — | — | 5·27 | 2·91 | 0·88 | 1·48 | — |
| 14.1.29 | ++ | ++ | ++ | + | — | — | 5·56 | 3·30 | 0·78 | 1·48 | — |
| 21.1.29 | ++ | ++ | ++ | + | — | — | 6·08 | 3·57 | 0·88 | 1·63 | — |
| 28.1.29 | ++ | ++ | + | — | Given Naganol 10 gm. intravenously | | | | | | |
| 4.2.29 | ± | ± | ? | — | — | — | 6·10 | 3·59 | 0·88 | 1·63 | — |
| 12.2.29 | + | ± | ± | — | — | — | 6·16 | 3·61 | 1·10 | 1·45 | — |
| 18.2.29 | ± | ± | ± | — | — | — | 6·32 | 3·47 | 1·45 | 1·40 | — |
| 25.2.29 | ± | ± | — | — | — | — | 6·32 | 3·45 | 1·47 | 1·40 | — |
| 4.3.29 | ± | ? | — | — | — | — | Estimations not carried out | | | | |
| 20.3.29 | ± | — | — | — | — | — | 6·27 | 3·48 | 1·50 | 1·29 | — |

CAMEL No. 16.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumin | Pseudo-Globulin | Eu-Globulin | Microscopic Examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 8.11.28 | ± | — | — | — | — | — | 6·07 | 3·43 | 1·38 | 1·26 | — |
| 15.11.28 | — | — | — | — | — | — | 6·07 | 3·57 | 1·38 | 1·12 | — |
| 22.11.28 | ± | — | — | — | — | — | 6·12 | 3·53 | 1·40 | 1·19 | — |
| 24.11.28 | | | | Given | *Trypanosoma soudanense* | | | | | | |
| 3.12.28 | ± | — | — | — | — | — | 6·18 | 3·52 | 1·40 | 1·26 | — |
| 10.12.28 | + | ± | ? | — | — | — | 6·13 | 3·93 | 1·36 | 1·10 | — (9.3.28) |
| 17.12.28 | + | + | ± | ? | — | ± | 5·93 | 3·20 | 1·28 | 1·45 | — (20.3.28) |
| 24.12.28 | ++ | ++ | ++ | ± | ± | ± | 6·15 | 2·91 | 1·38 | 1·86 | — |
| 31.12.28 | ++ | ++ | ++ | ± | ± | + | 5·98 | 2·91 | 1·26 | 1·81 | — |
| 7.1.29 | ++ | ++ | + | ± | — | + | 5·42 | 3·13 | 0·78 | 1·51 | — |
| 14.1.29 | ++ | ++ | + | + | ± | + | 5·62 | 3·01 | 0·93 | 1·68 | — |
| 21.1.29 | ++ | ++ | + | + | ± | + | 5·69 | 3·13 | 0·90 | 1·66 | — |
| 28.1.29 | | | Given | Naganol 10 gm. intravenously | | | | | | | |
| 4.2.29 | ++ | ++ | ++ | ± | ± | + | 5·64 | 3·13 | 1·00 | 1·51 | — |
| 9.2.29 | ++ | ++ | + | ? | — | + | 5·73 | 3·39 | 1·12 | 1·22 | — |
| | | Died | | | | | | | | | |

CAMEL No. 17.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumin | Pseudo-Globulin | Eu-Globulin | Microscopic Examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 8.11.28 | — | — | — | — | — | — | 6·28 | 3·57 | 1·51 | 1·20 | — |
| 15.11.28 | — | — | — | — | — | — | 6·20 | 3·57 | 1·51 | 1·12 | — |
| 22.11.28 | ± | — | — | — | — | — | 6·27 | 3·62 | 1·55 | 1·10 | — |
| 3.12.28 | + | + | ? | Given | *Trypanosoma soudanense* | | | | | | |
| 8.12.28 | | Died. | Trypanosomes swarming in blood | | | | 6·22 | 3·70 | 1·50 | 1·02 | — |
| | | | | | | | | | | | +++ |

CAMEL No. 19.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumin | Psuedo-Globulin | Eu-Globulin | Microscopic Examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 8.11.28 | — | — | — | — | — | — | 6·60 | 3·85 | 1·68 | 1·07 | |
| 15.11.28 | — | — | — | — | — | — | 6·46 | 3.85 | 1·61 | 1·00 | |
| 22.11.28 | ± | — | — | — | — | — | 6·50 | 3·83 | 1·65 | 1·02 | |
| 24.11.28 | | | Given *T. soudanense* | | | | | | | | |
| 3.12.28 | ± | — | — | — | — | — | 6·63 | 3·90 | 1·70 | 1·03 | |
| 10.12.28 | + | + | ± | ± | — | — | 6·49 | 3·91 | 1·51 | 1·07 | Tryps.present from 12/12 to 20/12 but *not* every day |
| 17.12.28 | + | + | ± | ? | — | — | 6·27 | 3·71 | 1·36 | 1·20 | |
| 24.12.28 | ++ | ++ | + | ± | ? | ± | 5·93 | 3·13 | 1·12 | 1·68 | |
| 31.12.28 | ++ | ++ | ++ | + | ± | — | 6·03 | 3·15 | 1·12 | 1·76 | |
| 3.1.29 | | | Given Naganol 10 gm. intravenously | | | | | | | | |
| 7.1.29 | ++ | ++ | ++ | + | ± | — | 5·01 | 2·00 | 1·20 | 1·81 | |
| 12.1.29 | | Died | allowed to get too ill before treating) | | | | | | | | |

CAMEL No. 20.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumiin | Pseudo-Globulin | Eu-Globulin | Microscopic Examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 8.11.28 | — | — | — | — | — | — | 6·05 | 3·85 | 1·20 | 1·00 | — |
| 15.11.28 | — | — | — | — | — | — | 6·09 | 3·71 | 1·26 | 1·12 | — |
| 22.11.28 | ± | — | — | — | — | — | 6·09 | 3·75 | 1·24 | 1·10 | — |
| 24.11.28 | | | | | Given *T. soudanense* | | | | | | |
| 3.12.28 | ± | ? | — | — | — | — | 5·98 | 3·62 | 1·26 | 1·10 | — |
| 10.12.28 | + | ± | — | — | — | — | 6·16 | 4·07 | 1·09 | 1·00 | — |
| 17.12.28 | + | ± | ± | ? | — | — | 6·63 | 3·85 | 1·05 | 1·73 | — |
| 24.12.28 | ++ | ++ | + | + | — | — | 6·23 | 3·30 | 1·20 | 1·73 | + daily from 18th-22nd. |
| 31.12.28 | ++ | ++ | + | ± | — | — | 6·18 | 3·16 | 1·26 | 1·76 | + |
| 7.1.29 | ++ | ++ | + | ± | + | — | 6·24 | 2·61 | 1·00 | 1·63 | — |
| 14.1.29 | ++ | ++ | + | + | Given Naganol (10 gm. intravenou , y) ± | — | 5·31 | 3·02 | 0·88 | 1·41 | — |
| 17.1.29 | | Died (cause apparently some poisonous plant in the green forage and not trypanosomiasis). | | | | | | | | | |

CAMEL No. 21.

| Date | Mercuric Chloride | | | | Water | Formol Gel | Total Protein | Albumin | Pseudo-Globulin | Eu-Globulin | Microscopic Examination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-15,000 | 1-20,000 | 1-25,000 | 1-50,000 | | | | | | | |
| 8.11.28 | — | — | — | — | — | — | 6·21 | 3·71 | 1·38 | 1·12 | — |
| 15.11.28 | — | — | — | — | — | — | 6·28 | 3·71 | 1·39 | 1·18 | — |
| 22.11.28 | — | — | — | — | — | — | 6·20 | 3·69 | 1·35 | 1·16 | — |
| 24.11.28 | | | | Given | *T. soudanense* | | | | | | |
| 3.12.28 | ± | ± | — | — | — | — | 6·17 | 3·73 | 1·36 | 1·08 | — |
| 10.12.28 | + | + | ± | — | — | — | 6·09 | 3·85 | 1·12 | 1·12 | — |
| 17.12.28 | + | + | ± | ? | — | — | 5·91 | 3·02 | 1·26 | 1·63 | ++ |
| 24.12.28 | ++ | ++ | ++ | ± | — | — | 5·78 | 2·91 | 1·26 | 1·61 | — |
| 30.12.28 | ++ | ++ | ++ | ± | — | — | 5·81 | 3·00 | 1·20 | 1·61 | +++ |
| 30.12.28 | Died suddenly, blood swarming with trypanosomes | | | | | | | | | | |

The changes in the globulins appeared in all cases to lag behind the results of the mercuric chloride test, an observation that seems to show that the flocculation tests give more sensitive indications of slight quantitative changes in the serum proteins than the actual analyses are capable of demonstrating. However, the possibility of a qualitative change in the globulin fractions must also be kept in mind.

A noteworthy observation is that positive reactions to the formol-gel test as applied to camels (gelation being accepted as the diagnostic phenomenon) did not develop parallel with relative increases in the eu-globulin. For example, in camels Nos. 17 and 20 it remained negative, in No. 19 a " partial " reaction was recorded on one occasion only in spite of an appreciable eu-globulin excess, while in No. 15 a positive formol-gel reaction developed before any increase in eu-globulin was demonstrable and became negative at the period of eu-globulin's greatest excess. In only one camel—No. 16—did the formol-gel readings run parallel with the others.

In this connection the fallacy of accepting gelation as a diagnostic phenomenon in the case of human kala-azar has been sufficiently indicated by other workers, while in the case of camel trypanosomiasis it seems that no feature of the formol-gel test is very reliable (a paper on this subject is in course of preparation by one of us, S.C.J.B.).

During the course of this investigation two valuable papers appeared on the formol-gel test and serum changes in human kala-azar cases by Lloyd and Paul [4, 5]. These investigators showed that in well established infections a most dramatic rise in the eu-globulin took place chiefly at the expense of the albumin. During treatment and cure very typical changes occurred in the various protein fractions before normal figures were reached. They concluded that the " fundamental cause of the formol leuco-gel reaction in kala-azar is the eu-globulin or substances precipitated with it present in the disease."

The danger of drawing comparisons between results in animals of different species is obvious, but several interesting points are common to both sets of results. Both the infecting organisms belong to the family Trypanosomidæ, in both the rise in eu-globulin is a characteristic change, and it may be noted that the results of our more chronic cases approach quite closely to their findings. This might be expected, as serological changes in kala-azar only develop when the infection has become well established. Napier reports that fully positive formol-gel reactions are seldom found in less than five months from the commencement of the disease.

A further parallel between the two infections is the fairly rapid return to normal of the protein fractions after treatment (in our case only one individual is available for discussion—Camel No. 15).

The chief criteria of value of a flocculation test are :—

(1) Sensitivity in detecting early serum changes.

(2) A clear cut border line between normal and diseased sera.

(3) Specificity in relation to disease.

The mercuric chloride test appears to conform to the first and

second of these essentials, while, in regard to the third, the extreme rarity in the camel of other infections which might possibly produce a similar reaction renders the test almost if not quite specific for trypanosomiasis.

SUMMARY.

1. The previous observations on the delicacy of the mercuric chloride test are confirmed.

2. Positive reactions are referable to the relative and absolute increase of the eu-globulin of the serum.

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[4]Lloyd and Paul. Serum changes in Kala-Azar. *Ind. Jl. Med. Res.*, 1928, Vol. xvi, No. i, p. 203.
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## BLACKQUARTER IN NIGERIA.

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THIS disease was diagnosed microscopically at the Laboratory in October, 1928. It had not previously been confirmed microscopically in Nigeria.

In the Hausa language the disease is known to natives as Arbau or Harbindəji. Reports of a disease simulating blackquarter have been received from several other parts of the country, and it is probable that the disease is widespread and of considerable economic importance. In the Plateau Province it has occurred very frequently, and during the last four months over twenty-five thousand cattle have been vaccinated.

The symptoms of the disease are frequently overlooked by the native owners, and such sudden deaths as usually occur in blackquarter are frequently attributed by them to juju or spirits.

Since considerable differences of opinion appear to exist concerning various points amongst workers with *Clostridium chauvei*, some of our experimental results, obtained when evolving a suitable vaccine for the disease, are interesting.

In natural outbreaks in Nigeria the disease appears not to be confined to animals between a few months and three years old, animals

much older being equally susceptible. The mortality in affected herds in the Plateau Province outbreak has been extremely heavy, some exhibiting a mortality of 25 per cent.

The diagnosis of the disease was based on the post-mortem picture, rabbit and guinea-pig inoculations, and cultural results. The rabbits used throughout did not react to the inoculum.

Deaths occurred in guinea-pigs in 24 to 72 hours, the inoculation consisting usually of 1 c.c. of culture given subcutaneously in the abdominal region. A guinea-pig post-mortem was considered typical when the results obtained were as follows :—

An emphysematous, œdematous, swelling extending over the abdomen and inferior surface of the thorax, the muscular tissue of these areas inflamed and black in colour, the pectoral muscles being particularly affected ; normal appearance of the intestines ; bacilli demonstrable in smears from the peritoneal surface of the liver, frequently arranged in pairs at an angle of 60° to each other ; bacilli recoverable in pure culture from the heart blood and bone marrow.

*Cultural Results.*

Literature on culture work with *Cl. chauvei* shows that the various authors have obtained various results with certain media. For example, Gaiger[1] reports good growth in liver bouillon, while McEwen[2] failed to grow the organism in the liver broth. Again, Gaiger reports growth on deep agar containing 1 per cent. glucose, whereas blackquarter strains isolated by Viljoen and Schreuber[3] did not grow on glucose agar.

Our cultural results were as follows :—

1 per cent. glucose plain broth—No growth.
1 per cent. saccharose plain broth—No growth.
Milk—Acid-congulation imperfect.
1 per cent. glucose agar—No growth.
1 per cent. glucose liver agar—Growth slow, and occurred in three days as minute dewdrop-like colonies on the surface of the media.

Martin bouillon—growth rather slow, characterised by a cloudiness of the media occurring in about 48 hours.

1 per cent. saccharose Martin bouillon—Good growth in 20 hours. This medium, as reported by the Kabete Laboratory[4], constitutes a suitable medium for vaccine preparation. It obviates the necessity for including solid tissues and enables one to obtain the bacteria free from tissue débris.

1 per cent. glucose liver broth—Good growth in twenty-four hours. Sporulation marked in 48 hours.

Hibler's medium.—Very good rapid growth but unsuitable for general vaccine production owing to tissue débris.

Tarozzi's medium—Growth similar to that on Hibler's medium.

During our cultural work we considered it of interest to attempt one of the experiments carried out by Scott[5] on the action of phenol

on cultures of *Cl. chauvei*. A small experiment enabled us to confirm the result obtained by Scott.

Phenol, sufficient to make a 1 per cent. solution, was added to a 48 hours growth of the bacillus on Hibler's medium. After 24 hours incubation subcultures were made to Hibler's media. These cultures were positive after 24 hours incubation and produced typical blackquarter lesions when inoculated into guinea-pigs.

*Staining.*

The reaction to Gram's stain was variable. Carbol-fuchsin for two minutes was generally used. The following method proved excellent for sporulating forms :—

Hot carbol-fuchsin three minutes.
Decolorise with 1 per cent. H2 S04.
Counter stain with methylene blue.

*Immunisation.*

Formalised vaccine was used as immunising agent. The age of the culture used was usually 24 to 48 hours.

A preliminary test was carried out on guinea-pigs. A 48 hour saccharose liver broth culture sterilised by the addition of ·5 per cent. formalin was used as vaccine. Four guinea-pigs were used for the test, one acting as a control. One of the guinea-pigs which received ·5 c.c. vaccine and the control both died from blackquarter when subjected to the test inoculation. The remaining two guinea-pigs, which received 1·5 c.c. and 1 c.c. of vaccine respectively, survived the test inoculation.

For Guinea-pig 8 the material appeared to be protective after being formalised for only $1\frac{3}{4}$ hours.

Table I.

| *Animal* | *Antigen* | *Dose* | *Number of days between Vaccination and Inoculation* | *Virus* | *Result* |
|---|---|---|---|---|---|
| Guinea-pig 3 | Vaccine formalised 5 hours | 1·5 c.c. | 19 days | 1 c.c. s/c | Slight reaction |
| Guinea-pig 8 | Vaccine formalised $1\frac{3}{4}$ hours | 1 c.c. | 17 days | 1 c.c. s/c | Reaction. Recovered |
| Guinea-pig 6 | Vaccine formalised 48 hours | ·5 c.c. | 17 days | 1 c.c. s/c | Died blackquarter |
| Guinea-pig 22 | Control | | | 1 c.c. s/c | Died blackquarter |

Utilising sheep, a second test was then carried out. Four sheep were inoculated subcutaneously with 1 c.c. vaccine, formalised four days. Twenty-three days later these sheep, a control sheep, and a guinea-pig received 1 c.c. of *Cl. chauvei* culture intramuscularly.

The following day a second control sheep received 1 c.c. of a subculture from the above. The four vaccinated sheep and the first

control sheep became lame but recovered. The control guinea-pig and the second sheep died from blackquarter. The fact that the first control sheep survived the test inoculation rendered this test somewhat inconclusive.

TABLE II.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus dose* | *Result* |
|---|---|---|---|---|---|
| Sheep 57 | Culture formalised 4 days | 1 c.c. | 23 days | 1 c.c. I/Mus. | Lame. Recovered |
| Sheep 54 | | 1 c.c. | 23 days | 1 c.c. I/Mus. | Lame. Recovered |
| Sheep 58 | | 1 c.c. | 23 days | 1 c.c. I/Mus. | Lame. Recovered |
| Sheep 55 | | 1 c.c. | 23 days | 1 c.c. I/Mus. | Lame. Recovered |
| Sheep 60 | Control | — | — | 1 c.c. I/Mus. | Lame. Recovered |
| Guinea-pig 24 | Control | — | — | 1 c.c. I/Mus. | Died blackquarter |
| Sheep 59 | Control | — | — | 1 c.c. subculture | Died blackquarter |

TABLE III.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus* | *Reaction* |
|---|---|---|---|---|---|
| Guinea pig 26 | Formalised 30 days | 1 c.c. | 18 days | 1 c.c. Culture subcuta. | Nil. |
| Guinea-pig 27 | | 1 c.c. | 18 days | 1 c.c. subcuta. | Nil. |
| Guinea-pig 29 | — | — | Control | 1 c.c. subcuta. | Died blackquarter |
| Sheep 62 | | 1 c.c. | Died Pleurisy | — | — |
| Sheep 61 | | 1 c.c. | 18 days | 1.5 c.c. Intra-M. | Nil. |
| | | | | 3·5 c.c. Intra-M. Six days later | Nil. |
| Sheep 66 | | | Control | 1·5 c.c. Intra-M. | Nil. |
| | | | | 3·5 c.c. Intra-M. Six days later | Died blackquarter |

A test was now carried out to ascertain if culture formalised for 30 days at room temperature remained protective against inoculation of virulent culture.

A 48 hour saccharose liver culture was sterilised by the addition of ·5 per cent. formalin and left in the culture cupboard for 30 days. Guniea-pigs 26 and 27 and sheep 61 and 62 then received 1 c.c. of

this vaccine subcutaneously. Sheep 62 died from pleurisy eleven days subsequent to inoculation. Eighteen days after receiving vaccine, guinea-pigs 26 and 27 and sheep 61 and 62 received 1 c.c. of culture subcutaneously. No. 29 died from blackquarter. Nos. 26 and 27 survived.

On the same day sheep 61 and sheep 66, the latter a control, received 1·5 c.c. of culture intramuscularly. Both animals failed to react. Six days after this inoculation they therefore received 3·5 c.c. of culture intramuscularly. The control sheep No. 66 died from blackquarter. No. 61 did not react.

In view of the fact that 1 c.c. virulent culture failed to infect sheep in experiment II and 1·5 c.c. in experiment III, a further experiment was carried out, using a larger dose of virus.

Sheep 65, 68, 69, and 70 received subcutaneously 1 c.c of vaccine formalised for 30 days. Guinea-pigs 30 and 31 received a similar inoculation. No. 65 died from pneumonia, as did also No. 70.

TABLE IV.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus* | *Reaction* |
|---|---|---|---|---|---|
| Sheep 65 | *Chauvei* culture formalised 30 days | 1 c.c. | | | Died pneumonia |
| Sheep 68 | | 1 c.c. | 24 | 2·5 c.c. Intramus. | Nil. |
| | | | | 3·5 c.c. Intramus. | |
| | | | | 5 days later | Lame. Recovered |
| Sheep 69 | — | 1 c.c. | 24 | 2·5 c.c. Intramus. | Nil. |
| | | | | 3·5 c.c. Intramus. | Recovered five days later |
| Sheep 70 | — | 1 c.c. | | | Died pneumonia |
| Sheep 71 | — | — | Control | 2·5 c.c. Intramus. | Nil. |
| | | | | 3·5 c.c. Intramus. | Died blackquarter |
| | | | | 5 days later | |
| Sheep 72 | — | — | Control | 2·5 c.c. Intramus. | Nil. |
| | | | | 3·5 c.c. Intramus. | Very lame and ill. |
| | | | | 5 days later | Recovered |
| Guinea-pig 30 | Formalised 30 days | 1 c.c. | 24 | 1 c.c. Subcut. | Died blackquarter |
| Guinea-pig 31 | Formalised 30 days | 1 c.c. | 24 | 1 c.c. Subcut. | Died blackquarter |
| Guinea-pig 35 | — | — | Control | 1 c.c. Subcut. | Died blackquarter |
| Guinea-pig 36 | — | — | Control | 1 c.c. Subcut. | Died blackquarter |

Twenty-four days after receiving vaccine numbers 69 and 70 and two controls, Nos. 71 and 72, were inoculated intramuscularly with 2·5 c.c. culture 72 days old. Guinea-pigs 30 and 31 and two controls, numbers 35 and 36, were inoculated subcutaneously on the same day with 1 c.c. of the same culture. None of the sheep reacted to the inoculation. All the guinea-pigs died from blackquarter. Five days later the sheep received 3·5 c.c. virulent culture intramuscularly. The control No. 72 died from blackquarter. Control 71 became very ill, being unable to rise for several days, but recovered. The two vaccinated sheep became slightly lame but recovered.

The result of our experience so far would appear to indicate that some native sheep have a resistance to artificial inoculation and that at least 3·5 c.c. virulent culture should be used as a test dose.

The results of the guinea-pig inoculations in this experiment are confusing, but are very similar to some of the results obtained by McEwen[2], who says " It was not infrequently found that the control animals lived while deaths occurred among those one had hoped to find protected by the previous inoculation of an antigen of proved value."

TABLE V.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus* | *Reaction* |
|---|---|---|---|---|---|
| Sheep 177 | *C. chauvei* culture formalised ·4 % for 7 days | 4 c.c. | 20 | 4 c.c. Intramus. | Lame. Recovered |
| Sheep 178 | | 4 c.c. | 20 | 3 c.c. Intramus. | Lame. Recovered |
| Sheep 179 | | 4 c.c. | 20 | 2 c.c. Intramus. | Lame. Recovered |
| Sheep 182 | — | — | Control | 2 c.c. Intramus. | Lame. Recovered |
| Sheep 176 | — | — | Control | 4 c.c. Intramus. | Died blackquarter |
| Guinea pig 1 | Culture formalised ·4% 7 days | 2 c.c. | 20 | 2 c.c. Subcut. | Lived. |
| Guinea pig 2 | | 2 c.c. | 20 | 2 c.c. Subcut. | Died blackquarter |
| Guinea-pig 3 | — | 2 c.c. | Control | 2 c.c. | Died blackquarter |
| Guinea-pig 2 | — | — | Control | 2 c.c. | Died blackquarter |
| Sheep 183 | Culture formalised ·4% 7 days | 4 c.c. | 29 | 4 c.c. Intramus. | Lame. Recovered |
| Sheep 184 | | 4 c.c. | 29 | 4 c.c. Intramus. | Lame. Recovered |
| Sheep 181 | | 4 c.c. | 29 | 4 c.c. Intramus. | Very severe reaction. Died in seven days. |
| Sheep 186 | — | — | Control | 4 c.c. Intramus. | Died blackquarter |
| Sheep 185 | — | — | Control | 4 c.c. Intramus. | Died blackquarter |

A further test was carried out on sheep and guinea-pigs. Six sheep, Nos. 179, 184, 178, 177, 183 and 181, received subcutaneously 4 c.c. culture formalised for seven days. Two guinea-pigs, Nos. 1 and 2, received 2 c.c. of the same vaccine. Twenty days afterwards sheep 177, 178, 179, and two controls received various doses of a virulent culture. The three vaccinated sheep and one control which received only 2 c.c. of culture became lame but recovered. The other control, which received 4 c.c. virulent culture, died from blackquarter. On the same day the two vaccinated guinea-pigs and two control guinea-pigs received 2 c.c. virulent culture. Both controls and one of the vaccinated guinea-pigs died from blackquarter.

Twenty-nine days after vaccination sheep 181, 183, 184 and two controls received 4 c.c. culture intramuscularly. The two controls died in twenty-four hours from blackquarter. Sheep 181 gave a very severe reaction to inoculation and died in seven days, when the local lesion appeared to be diminished. No *Cl. chauvei* organisms were demonstrable in smears from the liver surface. The remaining two vaccinated sheep became very lame but recovered.

The promising results obtained with sheep now enabled us to test the vaccine on cattle. The culture used was formalised for 30 days.

To part of the vaccine was added 20 per cent. of sterile ground tapioca to ascertain if any advantage occurred in increased immunity production by the addition of this substance.

Eight native heifers (Batch 1), approximate age two years, were inoculated subcutaneously with 10 c.c. vaccine.

The local reaction invariably consisted of a hard painful swelling, approximately 4-ins. square, appearing in 48 hours and disappearing in about two weeks. No temperature reactions occurred. Four similar bovines (Batch 2) received subcutaneously 10 c.c. vaccine with 20 per cent. sterile ground tapioca added two hours before use. The reactions were similar to the above.

Twenty-six days later, three out of Batch 1, one from Batch 2, and one control, received 10 c.c. virulent culture intramuscularly in the right gluteal region. One of the vaccinated became slightly lame but recovered. The remaining three gave no reaction. The control died from blackquarter in 84 hours.

Thirty-four days after vaccination four bovines from Batch 1, two from Batch 2, and one control, were inoculated intramuscularly in the left gluteal region with 10 c.c. virulent culture. None of the vaccinated animals gave any reaction to the inoculation.

The control died in five and a half days from typical blackquarter, a very severe local lesion being produced.

The two remaining vaccinated bovines are retained in order to test the duration of immunity produced.

TABLE VI.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus* | *Reaction* |
|---|---|---|---|---|---|
| Bovine 1 | Culture formalised ·4% for 30 days | 10 c.c. | 26 | 10 c.c. I/M | Lame. Recovered |
| Bovine 2 | ,, ,, | 10 c.c. | 26 | 10 c.c. I/M | Nil. |
| Bovine 3 | ,, ,, | 10 c.c. | 26 | 10 c.c. I/M | Nil. |
| Bovine 4 | ,, ,, plus 20% tapioca | 10 c.c. | 26 | 10 c.c. | Nil. |
| Bovine 5 | — | — | Control | 10 c.c. I/M | Died blackquarter |
| Bovine 6 | Culture formalised for 30 days | 10 c.c. | 34 | 10 c.c. I/M | Nil. |
| Bovine 7 | ,, ,, | 10 c.c. | 34 | 10 c.c. I/M | Nil. |
| Bovine 8 | ,, ,, | 10 c.c. | 34 | 10 c.c. I/M | Nil. |
| Bovine 9 | ,, ,, | 10 c.c. | 34 | 10 c.c. | Nil. |
| Bovine 10 | ,, ,, plus 20% tapioca | 10 c.c. | 34 | 10 c.c. I/M | Nil. |
| Bovine 11 | plus 20% tapioca | 10 c.c. | 34 | 10 c.c. I/M | Nil. |
| Bovine 12 | — | — | Control | 10 c.c. | Died blackquarter |
| Bovine 13 | Culture formalised ·4% 30 days | | | | |
| Bovine 14 | ,, ,, plus 20% tapioca | | | | |

TABLE VII.

| *Animal* | *Antigen* | *Dose* | *Days between Vaccination and Test* | *Virus* | *Reaction.* |
|---|---|---|---|---|---|
| Bovine 1 | Culture formalised | 10 c.c. | 33 | 10 c.c. I/M | Nil. |
| Bovine 2 | ·4% for 27 days | 10 c.c. | 33 | 10 c.c. I/M | Nil. |
| Bovine 3 | ,, ,, | 5 c.c. | 33 | 10 c.c. I/M | Nil. Nil. |
| Bovine 4 | ,, ,, | 5 c.c. | 33 | 10 c.c. I/M | Nil. |
| Bovine 5 | — | — | Control | 10 c.c. I/M | Died blackquarter |

An attempt was now made to test the efficacy of a reduced dose of the vaccine. The culture used was formalised for 27 days.

Five native heifers were inoculated subcutaneously with 5 c.c. of this vaccine (Batch 1). Three native heifers were inoculated with

10 c.c. (Batch 2). Local reactions were similar to those in the previous experiment. No temperature reactions occurred.

Thirty-three days later two from each batch received 10 c.c. virulent culture intramuscularly in the right gluteal region. One control received a similar inoculation. The vaccinated animals exhibited no reaction to the inoculum. The control died from blackquarter in eight days, the post-mortem being typical.

The remainder have not yet been tested but it would appear that 5 c.c. of vaccine may prove to be a convenient dose for field use.

The results of the test inoculations carried out so far may be tabulated as follows :—

| | *Vaccinated* | | *Controls* | |
|---|---|---|---|---|
| | *Lived* | *Died* | *Lived* | *Died* |
| Bovines .. .. | 14 | 0 | 0 | 3 |
| Sheep .. .. | 12 | 1 | 3 | 6 |
| Guinea-pigs .. | 5 | 4 | 0 | 6 |

*Preparation of Vaccine for Field Use.*

At present our routine method of preparation of the vaccine for use in the field is as follows :—

The medium consists of Liebig's broth plus 1 per cent. peptone, 10 per cent. fresh ox brain, and 1 per cent. sugar (glucose or saccharose), *p*H 8.

The brain tissue minus the sugar is autoclaved at 25 lbs. pressure the first day, and then 15 lbs on each of two successive days.

The sugar is sterilised at 5 lbs. pressure and then added to the brain medium under sterile precautions.

The medium is made up in four litre flasks and remains in the incubator at 37° for 24 hours prior to inoculation.

The inoculum consists of heart blood from guinea-pigs dead of blackquarter subsequent to inoculation with known blackquarter muscle emulsion. The flasks are then incubated for 48 hours. At the same time a few tubes of media containing 1 per cent. saccharose and a few containing 1 per cent. salicin are inoculated to complete the diagnosis.

Formalin is then added to make ·4 per cent. and the flasks are allowed to stand for at least 48 hours. The vaccine is then tested for sterility by guinea-pig inoculation and inoculation of small tubes of aerobic and anærobic media.

Prior to bottling the fluid is decanted from the flasks, leaving the brain tissue behind.

We have found that the brain medium gives a better growth than the saccharose Martin bouillon and no elaborate anærobic precautions are necessary.

## Summary.

1.—Blackquarter in Nigeria attacks animals of all ages with equal facility.

2.—One per cent. phenol does not inhibit the growth of *Cl. chauvei* in culture.

3.—Some native sheep appear to offer resistance to artificial infection with local strains of blackquarter.

4.—Culture sterilised with ·4 per cent. formalin and inoculated subcutaneously completely protects bovines against subsequent intramuscular inoculation with virulent culture.

5.—The preparation of this formalised culture is described.

I am indebted to Captain W. W. Henderson, D.V.S.M., Chief Veterinary Officer, for permission to publish these notes.

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## NOTE ON THE TREATMENT OF AN INFECTION WITH *B. abortus* IN RABBITS.

By Cecil I. B. Voge, B.Sc., Ph.D.
(*Carnegie Research Scholar.*)
(*From the Animal Breeding Research Department, University of Edinburgh.*)

In previous papers [1, 2] it has been shown that the changes which occur in blood serum obtained from syphilitic individuals or from cattle suffering from an injection of *B. abortus*, are associated with the serum euglobulin fraction.

In a later communication[3] a method was devised whereby isolation of a serum fraction rich in such a reacting substance could be effected.

The work of Kroeger and Hektoen[4], Rosenholz[5], and Cambessédes and Cochez[6], criticised in the previous paper, bears out the feasibility of such separation and the possibility of making use of such a fraction for therapeutic purposes.

Owing to lack of space and the necessary funds for carrying out a satisfactory experiment on a large scale, a tentative experiment was devised in order to ascertain whether such a serum fraction was capable of protecting an animal against a subsequent infection with the specific organism.

The animals employed were rabbits, and the material used in treatment was (*a*) the water-insoluble serum protein fraction from a cow's serum, whose agglutinating titre against a suspension of *B. abortus*

was greater than 1 : 200.; (*b*) a similar serum fraction obtained from a cow whose agglutinating titre against a suspension of this organism was less than 1 : 20.

Owing to the small numbers of animals employed in these experiments the observations made have been as comprehensive as possible, so as to provide a reliable indication as to whether significance could be attached to the results.

Four rabbits were taken whose age was approximately six months. The animals were weighed at intervals of approximately one week.

*Rabbit* 1 ♂. This animal was used for mating and was untreated and uninfected ; until the 86th day it was not in contact with the infected does, but on that date he was mated to the control (No. 4, no treatment). It will be noted that a fall in weight occurred during the subsequent 14 days.

Whether this fact is significant and indicative of a slight infection is not certain.

*Rabbit* 2 ♀. This doe was injected intravenously at weekly intervals (·5, 1·0, 1·5, 2·0 c.c.) with the euglobulin fraction obtained from a cow whose blood serum gave an agglutinating titre greater than 1 : 200 with a suspension of *B. abortus*.

A week after the last injection this doe was infected (*vide infra*). A steady rise in weight occurred up to the 64th day of the experiment, and on the next day three full term rabbits were produced. These were either born dead or died shortly after birth. Cultures from stomach were negative to *B. abortus* and no lesions were observed. This doe afterwards lost weight until the 78th day and then steadily increased. Upon the 94th day she was again mated, and on the 120th day produced a healthy full term litter. Two of these died accidentally a couple of days later (one was dropped, and the mother apparently trampled upon the other). The other two and the mother at the end of the experiment were alive, healthy, and putting on weight. The agglutinating titre of this doe is 1 : 100.

*Rabbit* 3 ♀. This doe was injected in a similar manner to the preceding one, except that the serum used in preparation was from a normal cow. Nine days after injection weight was rapidly lost and abortion occurred. An organism microscopically, culturally, and immunologically similar to *B. abortus* was isolated. From the 58th day weight was gained, but in spite of frequent matings (other bucks were also used) this animal appears to be sterile as a result of the infection, although on two occasions she made a nest, which is thought by some to be a sign of ovulation. This sterility after an infection with *B. abortus* is sometimes encountered in cattle.[7]

The agglutinating titre of the blood serum of this doe is 1 : 100.

*Rabbit* 4 ♀. This rabbit acted as the main control and received injections of normal saline before injection. As a result of the first mating no visible abortion occurred. This is quite in keeping with the facts encountered in contagious abortion in cattle, in which the first slipping of the calf may occur so early in pregnancy that it may be unnoticed. This doe made a nest preparatory for the birth of the litter but nothing was observed.

Upon a second pregnancy being established she aborted a very miniature fœtus and she herself died the following day. An organism indistinguisable by usual tests from *B. abortus* was isolated from mother and fœtus. The agglutination titre of the former was 1 : 200.

Infection was caused by injecting intravenously 1 c.c. ($460 \times 10^6$ organisms). Two cultures were used at the commencement of previous work and since they appeared to be indistinguishable they were mixed. For one strain we are indebted to Professor Mackie, Department of Bacteriology, Edinburgh University.

The serum fractions employed were obtained by dialysing blood serum for 18 hours against distilled water, after which period of time most of the insoluble euglobulin has passed out of solution.

In the case of anti-serum it is found that the active agglutinating material is combined with this fraction. The nature of the combination, whether adsorption or not, cannot be discussed here and is a most debatable point. A full description of the method is given by Voge, in the Bichemical Journal[3].

Further experimentation upon these lines is found to be impossible since the author is obliged to confine himself to other problems. The results here recorded are emphatically only in the nature of preliminary work.

The experimental conditions in the present research have been unnecessarily severe, so that if promising results were shown under these circumstances further work would be warranted.

Firstly, the course of preventative inoculation might well have been extended both before and after infection, and, secondly, the method and dose of infection were somewhat drastic, and therefore boded ill for a successful termination to the experiment.

The normal route of infection is either by the mouth or the vagina, and hence the intravenous route may be expected to have more far reaching results.

However, even with such an outlook, it is considered that the results are full of promise.

## Conclusions.

Serum euglobulin obtained from anti-abortus cow serum appears to be capable of affording protection to a rabbit subsequently infected with *B. abortus*. Serum euglobulin from normal serum does not act in this manner, although the effects of such an infection appear to be less drastic than where no treatment at all is given.

My thanks are due to all those who enabled me to collect sufficient material from the City of Edinburgh Slaughter House, and more especially to Mr. Poole, of the Animal Diseases Research Association, who offered me scientific hospitality upon many occasions.

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## THE RELATIONSHIP BETWEEN UNDULANT FEVER AND EPIZOOTIC ABORTION.

As long ago as 1896, when the brilliant researches of Bang and Stribolt revealed the cause of epizootic abortion in cows and the fact that the disease was experimentally transmissible to other domesticated animals, there was reason to consider the question whether the human subject might also be susceptible to infection. Naturally, at that time it was supposed that if such infection actually took place evidence of it should be sought in connection with the occurrence of abortion in women, since abortion appeared to be the only clinical sign of infection in the lower animals. As no evidence pointing in that direction was forthcoming, it seemed either that the Bacillus abortus was not pathogenic for the human female, or that in ordinary circumstances opportunity for the infection of women seldom or never occurred, and that there was no reason why bovine contagious abortion should be added to the list of animal diseases communicable to man. There is now available evidence to prove that the bovine disease must be so enrolled, and it is interesting to observe that an error in nomenclature was partly responsible for the delay in recognising the fact. The error was that the causal organism of Mediterranean fever, discovered by Bruce, in 1887, was named the *Micrococcus melitensis*. It is now generally admitted that the organism in question is not a micrococcus, but the fact that it was so named tended to delay the discovery that the B. abortus was morpologically indistinguishable from it. That discovery was made by Evans, in 1902, but before that date other evidence incriminating the bovine bacillus as a cause of disease in man had come to light. It is now known that the resemblance between the two germs goes much deeper than their morphological character; it includes a similarity in cultural peculiarities, serological reactions, and pathogenic power for different animal species. At the present moment, the only matter that stands in doubt is whether the two organisms belong to the same species in the sense in which that word is applied in classifying the bacteria. And here it is advisable to take note of the fact that both the organisms have suffered a change of name, Micrococcus melitensis becoming *Brucella melitensis*, and Bacillus abortus *Brucella abortus*; and, further, that the human disease formerly termed Mediterranean or Malta fever is now known as Undulant fever.

Undulant fever has long been known as a disease of human beings prevalent in countries bordering on the Mediterranean Sea, and in respect of its etiology it first received fruitful investigation at the hands of an English Commission, in consequence of the serious incidence of the disease among the Marines at the Naval Station in Malta. This led to the discovery of the germ of the disease by Bruce, and of the fact that the disease was primarily one of goats, from which it was conveyed to human beings who drank raw milk. Undulant fever—

meaning thereby the disease in man—is a remittent fever, with a very characteristic temperature chart, from which it derives its new name. The visible illness may last for weeks or months, and it is fatal in about 3 per cent. of cases. Prevention of the use of raw goat's milk had an immediate and striking effect on the incidence of the disease in the British Garrison.

During recent years the disease appears to have made serious extensions, always connected with the spread of the infection among goats. Very important facts are that the disease is generally non-febrile in the goat, and that abortions are frequent in infected herds.

All these facts were for a time accepted without serious apprehension in other countries, because it was assumed that this undulant fever was an enzootic disease, affecting the goat only among the domesticated animals, and, consequently, that where goat's milk was not in use undulant fever in man was not likely to appear. This confidence was shaken when it appeared that in certain parts of the world cases of undulant fever in man had occurred independently of any disease in goats or the use of goats' milk. In a number of these cases serological and other tests agreed in showing that the illness of the patients was caused by the B. abortus—that is, the organism responsible for epizootic bovine abortion.

In a recent review (F. Huddleson) of the evidence of infection of human beings with the bacillus of contagious abortion it is stated that Malta fever is known to be endemic in the south-western part of the United States, and possibly in Mexico. The disease there is apparently identical with the one occurring in southern Europe in that it is contracted by drinking milk from infected goats. It has been found, however, that in the eastern, northern, and central States, cases have been occurring of a disease in human beings which runs a course resembling the genuine Malta fever, but is unconnected with the drinking of goats' milk. It is not known whether the disease is of recent occurrence, or has been in existence but not diagnosed for a number of years.

Orr and Huddleson investigated 33 cases of this disease occurring in man in different parts of Michigan, and found that there was no history that the patients had been associated with goats or ingested their products.

Many of the patients had a history establishing a connection with abortion in cows, or of drinking milk from infected herds. In 50 per cent. of the cases in which the history of the patient pointed to possible infection from a bovine source contagious abortion was found to exist in the suspected herd.

Unfortunately, recent investigations have furnished further very strong evidence that the cattle disease is not without danger to the human species. The most striking of these was carried out in Denmark, by Dr. Martin Kristensen*. While working at the State Serum Institute,

*Extract from Epid. Report ; Health Organisation, League of Nations, No. 14, 1928.

Kristensen thought it would be of interest to endeavour to discover whether any of the blood samples sent to the Institute from suspected cases of typhoid or enteric fever would, when tested, give evidence that the patient had been infected with the abortus. In the course of the year following April 1, 1927, 2,500 samples from about 2,150 patients were thus tested, and the result was the discovery of evidence that 222 of these patients had been infected with the B. abortus. The test applied to these samples was the agglutination test which is now everywhere employed for the diagnosis of contagious abortion in cows, and it must be understood that if the same result had been obtained with blood from cows it would not have been doubted that the cows in question had been infected with contagious abortion.

Out of the samples of blood from 2,150 patients, 172 gave evidence of infection with typhoid and 126 with paratyphoid B., as against the 222 in which the result indicated infection with the B. abortus. It therefore appeared that in Denmark infection with the abortion bacillus is actually more frequent than infection with the germs of typhoid or paratyphoid B. fever. In 5 of the 298 cases which gave the typhoid or paratyphoid reaction there was also agglutination of the B. abortus, but in these 5 cases the condition of the patients indicated that the illness was probably caused by the B. abortus alone. In all the other cases that gave a positive reaction for the B. abortus the test for typhoid or paratyphoid was negative. Further, and not less important, in 18 out of 27 cases in which a culture experiment with the blood was made B. abortus was found to be present. In the remaining nine cases no growth was obtained, but the symptoms presented by these were clinically the same as those presented by the 18 in which culture experiments were positive. Fifty samples of serum were received at the Institute from a district in Greenland where there are no cattle, and in all of these the test for evidence of infection with the B. abortus was negative. Among the persons suffering from infection with B. abortus 40 lived in Copenhagen, 34 in other towns, and 146 in rural districts. Information about the probable source of infection was available for 209 of the persons whose serum agglutinated B. abortus, and it showed that 171 had been exposed to risk of infection while attending cattle, or had the double risk of such attendance and drinking raw milk or cream from infected cows. Not more than one case occurred in the same family, and the cases were not specially numerous in any particular part of the country.

What these results prove is that in Denmark, also, there are occurring among human beings cases of febrile disease resembling the undulant fever of Southern Europe, and caused by the bacillus of cattle abortion, and investigations on similar lines have had the same results in the United States, Holland, and Sweden.

Lastly, in this connection, it must be mentioned that recently in England several cases of what appeared to be undulant fever have

been reported in which the disease must have been contracted locally.

The conclusion that must be drawn from the foregoing facts is that the disease of cattle termed contagious or epizootic abortion is communicable to man, in whom it takes a form indistinguishable from the undulant fever of the Mediterranean. Probably about 30 per cent. of all the cows in Europe are affected with the disease, and about 10 per cent. pass out abortion bacilli in their milk. The facts are serious, but need not occasion a panic. There has been no sudden change in the circumstances, but these have been rather suddenly apprehended.

It is comforting to know that even in the Malta garrison before any precautions were taken the incidence of the disease was less than 5 per cent. per annum, although 50 per cent. of the goats were infected and there is reason to believe that many of them yielded milk much more heavily charged with the bacilli than is usual in cows. There is also a possibility that the bacilli derived from cows are less virulent for man than those from goats.

In Sweden, undulant fever has already been made a notifiable disease, and the Ministry of Health may think it advisable to do the same here in order to discover to what extent the infection is being passed on from cattle to man in Great Britain. That is not yet known for any country, but there is no ground for hoping that it is less here than any of the countries already mentioned. Doubtless when attention is turned to the disease it will appear to become more frequent, and there will be a danger of hasty and ill-considered action. Meanwhile, housewives can be assurred that boiling of the milk is a perfectly certain means of prevention, and, indeed, a temperature much below the boiling point can be relied upon to destroy the bacilli.

At first sight it appears to be a very remarkable fact that it has taken more than thirty years after the discovery of their respective germs to prove the identity of contagious abortion in cattle with undulant fever in the goat and in man, but very little retrospection is necessary to show why the discovery was so long delayed. A contributory fact was the misnaming of the organism proved by Bruce to be the cause of Malta fever, for, after Bang's description of the B. abortus, that was almost certain to put " off the scent " anyone speculating as to whether the disease among the goats in Malta might be identical with contagious abortion ; and another was that the description of the cultural characteristics of the Malta fever germ was incomplete. But, most of all, the mere suspicion of the identity was prevented by the apparent fundamental clinical differences between contagious abortion and Malta fever as it affected the human subject. The bovine disease was without effect on the animals' general health, the human was a serious, and sometimes fatal, fever. The one was essentially a metritis, and the common method of infection was supposed to be copulation ; in the other, infection was mainly, if not exclusively, by ingestion.

# OBITUARY.

## THE LATE MAJOR-GENERAL SIR FREDERICK SMITH, K.C.B., C.M.G., Hon. Assoc. R.C.V.S.

By the death of Sir Frederick Smith, which occurred on July 27th last, the veterinary profession lost one of its most distinguished members.

Born in 1857, he entered the Royal Veterinary College as a student in 1874, and obtained the membership diploma of the Royal College of Veterinary Surgeons in 1876. In the same year he entered the Army Veterinary Service, being the last officer but two to join under the old regimental system. He was gazetted to the Royal Artillery in 1877, and to the 12th Royal Lancers in 1880. In 1886 he joined the staff of the Army Veterinary School, Aldershot, as Assistant Instructor and was made Professor in 1887. On the expiration of his period of office at the School he was appointed to the Remount Department. He served with the Nile Expedition in 1898, and in the South African War 1899-1902, and became P.V.O. South Africa in 1903. He was appointed Director-General in 1907, and retired in 1910. During the Great War he was re-employed on Home Service.

Sir Frederick was a prolific writer, and did much to enrich the literature of his profession. Putting aside the many valuable articles which he contributed to this and other professional journals, he was the author of four works of outstanding merit ; *viz.*, text-books dealing respectively with Veterinary Physiology and Veterinary Hygiene, a History of Veterinary Science (down to the end of the 18th Century), and a History of the Royal Army Veterinary Corps. Text-books are by their nature ephemeral, but the last two of these works will endure as a monument to the energy, erudition, sound judgment, and literary talent of their author.

Sir Frederick held stern views of life and duty. He appeared to be inspired by a determination to contribute his utmost to the advancement of veterinary science, and his intimate friends had often to observe with regret that his plan of work did not leave him even the minimum of time necessary for rest and recreation if health is to be preserved. With all his gifts, and the record of valuable work accomplished, he remained singularly modest with regard to his own abilities. A manner that was rather reserved tended to conceal his real temperament, which was warm and sympathetic. For many members of the profession his death has terminated a highly esteemed friendship.

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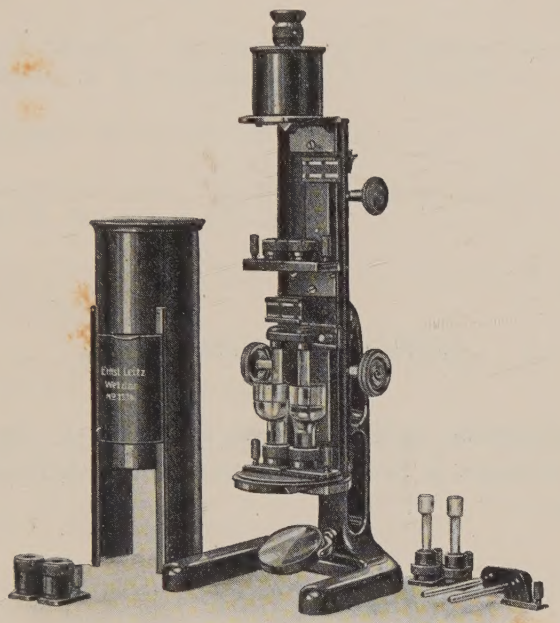


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# CONTENTS.

## GENERAL ARTICLES.


PAGE

Studies on Bovine Mastitis. The Bacteriology of Mastitis. By F. C. Minett, A. W. Stableforth, and S. J. Edwards ... ... 213

Nephritis in Animals. By Sir John McFadyean ... ... ... 231

Anærobic Infections in Lambs. By W. Lyle Stewart, M.R.C.V.S., and D. W. Henderson, B.SC. ... ... ... ... ... 241

Endemic Infection of Guinea-Pigs with B. ærtrycke (Mutton). By J. W. Edington ... ... ... ... ... ... ... 258

A Note on the Mercuric Chloride Test for Trypanosomiasis. By F. H. Manley, B.V.SC., M.R.C.V.S. ... ... ... ... ... 268

Paragangliomata of the Adrenal Medulla, with a Report of Three Cases in Cattle. By William Susman, B.A., M.D. (Q.U. CAN.); C. A. McGaughey, M.R.C.V.S., D.V.S.M. (VICT.); and H. L. Torrance, M.R.C.V.S., D.V.S.M. (VICT.) ... ... 269

A Note on Bovine Tuberculosis in Tropical Africa (British Cameroons). By F. H. Manley, B.V.SC., M.R.C.V.S. ... ... ... 276


## REVIEWS.


The Principles of Bacteriology and Immunity. By W. W. C. Topley, M.A., D.SC., F.R.C.P.; and G. S. Wilson, M.D., M.R.C.P., D.P.H. ... ... ... ... ... ... ... ... 279

Insects, Ticks, Mites, and Venomous Animals of Medical and Veterinary Importance. By Walter Scott Patton, M.B., CH.B. (EDIN.), F.R.S.; and Alwen M. Evans, D.SC. ... ... ... 279

The Essentials of General Physiology. By Eric Ponder, M.D., D.SC. ... 280

The Essentials of Histology. By Edward Sharpey Schafer, F.R.S. ... 280

Bainbridge and Menzies' Essentials of Physiology. Sixth Edition. Edited and revised by H. Hartridge, M.A., M.D., SC.D., F.R.S. ... ... ... ... ... ... ... ... 280

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